THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XXII MARCH, 1946 NUMBER 2

GYNECOMASTIA *

HOWARD T. KARSNER, M.D.

(From the Army Institute of Pathology, Army Medical Museum, Washington 25, D.C.)

The literature covering enlargement of the male breast has been reviewed splendidly up to 1930 by Kriss [1] and up to 1933 by Menville.[2] The condition was known to Aristotle. Galen first used the term "gynecomastia" (woman, breast) to indicate an increase in the amount of fat underlying the breast.[3] Gynecomastia, whether corresponding to Galen's conception or not, was described by Paulus Aegineta, Fabricius ab Aquapendente, Paracelsus, Haly Abbas and others of mediaeval times.

Definitions

Galen's definition applies only to what is now called pseudogynecomastia. Various authors have referred to gynecomastia in terms of the breast of females. For example, Weber [4] spoke of it as mammary feminism, and Cheatle and Cutler [5] defined it as "an affection of the male breast in which the gland tends to assume the size, shape and sometimes the functions of the female breast." Similar views as to the nature of the lesion have been expressed by Bronstein,[6] by Maciel Crespo,[7] by Ilabaca Leon,[8] and by Word and Reed.[9]

Fifty years ago, however, Stieda,[10] basing his definition partly on etymology, said that the term "gynecomastia" is justified only by external form and volume, that microscopically the tissue neither corresponds to nor closely simulates the functional breast of the female.

Other writers have referred to gynecomastia as hypertrophy of the breast; these include Deaver and McFarland,[11] Maliniac,[12] Dunn,[13] and others. It is listed in the Quarterly Cumulative Index under the heading "Hypertrophy of the Breast." Ewing [14] used the term "gynecomastism" to include hypertrophy of the male breast with or without changes in the testicle. Williams,[15] in one of the early discussions, said that gynecomastia differs from hypertrophy in the female in that there is no tendency to indefinite increase in size; growth ceases when it attains the dimensions of the normal breast of the female. Others, such

* Received for publication, May 10, 1945.

as Webster,[16] avoided controversy by referring to the condition simply as enlargement of the male breast.

Menville[2] said that much confusion has resulted from a failure to define clearly the limits of gynecomastia. It may be added that if the term "hypertrophy" merely means increase in size, it may be properly applied, but if it carries in addition the connotation of increased functional capacity, it may not be. The belief that the process duplicates the breast of the female is incorrect, as is the assumption that it represents feminization, if that term is used in its full meaning. Many men with gynecomastia have all other morphologic and functional evidence of masculinity (Figs. 1 and 2). Gynecomastia differs from adenofibroma, chronic cystic mastitis, mazoplasia, and other mammary diseases of the female. Further confusion is caused by failure to distinguish pseudogynecomastia, a deposition of fat in or under the breast, from the hyperplasia of true gynecomastia. Introduction of the term "anisogynecomastia" (Del Castillo, de la Balze, and Reforzo Membrives[17]) to indicate inequality of bilateral enlargement has done nothing to lessen the confusion. One of the purposes of this communication is to arrive at a definition which will separate gynecomastia from inflammatory diseases and neoplasia of the breast of the male.

Pathologic Anatomy

The altered mammary gland is described as a button-like or plate-like mass, or a nodule, under the nipple, rarely attached to the nipple, but not adherent to other structures. The mass may become fairly large, attaining a weight of as much as 600 gm., and appear as a more or less globular body. Several authors have stated that it may reach the size of the breast of a virgin—whatever that may be. The mass is firm, with smooth outline, and may be tender or painful, or both. During the process of growth, which goes on for a few months or years, the enlargement is ordinarily gradual, but in a few instances there may be periods of interruption or acceleration. After it reaches a certain size, growth ceases. Secretion occurs in a few cases, appearing either spontaneously or on expression, but this secretion has not been proved to be milk.

In most cases the nipple and areola show no departure from the normal. In a few there may be variable degrees of enlargement and projection of the nipple, widening of the areola, sometimes with increased pigmentation, and prominence of the glands of Montgomery.

The mass is firm, of smooth outline, clearly but not sharply defined; it offers considerable resistance to cutting and in cross section is firm, slightly bulging, moist, pale gray or bluish gray, fairly homogeneous, often translucent, and sometimes slightly lobulated. The mass merges

fairly abruptly with the surrounding adipose tissue, but is not definitely encapsulated. Even when considerably distended, the ducts are not visible in cross section, but in rare instances cysts a centimeter or more in diameter are seen.

The microscopic picture is not described as well as the gross in the literature. Gill [18] said that the mammary tissue resembles that of a girl 12 or 13 years old. Both Goodman [19] and Geschickter [20] compared the lesion to the breast of the female at puberty; zum Busch,[21] to the secreting mammary gland.

Other writers have compared gynecomastia to mammary disease in the female. Sullivan and Munslow [22] regarded the lesion as almost identical with chronic cystic mastitis of the female, more commonly of the adenosis type. Menville [2] could find no clear distinction between gynecomastia and fibro-adenoma of women, an opinion in which Lewin [23] concurred. Webster [16] made the confusing statement that gynecomastia is indistinguishable from fibro-adenoma and from chronic cystic mastitis, two lesions which can readily be differentiated. Gill [18] stated that gynecomastia resembles pericanalicular adenofibroma but without whorls of connective tissue. Lewis and Geschickter [24] were of the opinion that gynecomastia differs from adenoma in the absence of acini and lobules, but Erdheim [3] and also Cheatle and Cutler [5] described acini, and Bonn and Evans [25] spoke of imperfectly formed acini. No clearly identifiable acini were found in the material used in this study.

Multiplication of ducts and epithelial proliferation are well described. Erdheim [3] as well as Cheatle and Cutler [5] indicated that the larger ducts are not involved, but that is not true.

Stieda [10] (1895), Erdheim [3] (1928), and Menville [2] (1933) had stated that the increase in size of the breast is due principally to proliferation of connective tissue stroma; but Cheatle and Cutler [5] attributed it to an increase in volume of the entire tissue of the gland. Moore, Wattenberg, and Rose [26] drew attention to the great increase in connective tissue, intimating that it was partially due to edema. Among the specimens in this survey edema was infrequent, the connective tissue bulky, and the periductal tissue loosely arranged.

The presence of lymphocytes, plasma cells, and large mononuclear cells in the periductal tissue and sometimes in the dense stroma has caused little comment. Ingleby [27] described an infiltration of lymphocytes and an occasional plasma cell around many ducts. Menville [2] reported a lymphocytic infiltration with some plasma cells and eosinophils in loose periductal tissue, where Geschickter [20] also noted a moderate number of wandering cells. In photomicrographs incorporated in other publications cellular infiltration is often observed but not referred to in the text.

Present Study

This study was undertaken at the request of the Director of the Army Institute of Pathology, Army Medical Museum, Col. J. E. Ash, M.C., U.S.A., and his associate, Lt. Col. Balduin Lucké, M.C., A.U.S. It is based on protocols and microscopic sections from 284 cases of gynecomastia, nearly all from soldiers, filed in the Army Medical Museum, from the Zone of the Interior and Theaters of Operation. Since beginning this investigation many new cases have been filed but not added to the material utilized as it already represented a fair sampling of the whole collection.

GENERAL DATA

Data on various clinical aspects were kindly collected by Mrs. Helen Friedman. The information in the protocols is not complete in many cases, but is extensive enough to indicate the characteristics of gynecomastia.

Age. The relation of the lesion to age is suggested by classifying by decades the 280 cases in which the patients' ages were recorded.

Age by decades	10–19	20–29	30–39	40–49	50–59	60 and above
Number of cases	44	192	23	12	7	2

The youngest patient was 14 years old, the two above 60 were 76 and 77 years of age.

Race. Of the 228 patients whose race was noted, 193 were white, 32 Negro, 1 each Puerto Rican, American Indian, and Chinese.

In regard to both age and race, the figures do not represent accurately the distribution in the civilian population, and they cannot be corrected statistically because data are not available.

Breast Involved. Of the 274 cases in which the location of the lesion was defined, both breasts were affected in 12, the right alone in 138, and the left alone in 124.

The location of gynecomastia has been tabulated in relation to racial distribution as follows:

Race	Breast Involved		
	Right	Left	Bilateral
White	87	79	10
Negro	15	14	
Unspecified	36	29	2
Puerto Rican		1	
Chinese		1	
American Indian*			
Totals	138	124	12

* In the case of the American Indian the side involved was not stated.

Duration of Complaint. From the protocols it is impossible to state in most instances whether the history began with mammary enlargement, pain, tenderness, or trauma. Two patients claimed to have noticed the lesion only 1 week before operation. The following table gives the duration according to the histories:

Duration	Less than 3 mos.	3 mos. to less than 6 mos.	6 mos. to less than 1 yr.	1 yr. to less than 2 yrs.	2 yrs. and more
Number of cases	90	38	27	19	62

Among soldier patients there is the possibility that the matter of service-connected disability may at times distort the histories. More dependable information may be gathered from histories which date back 2 years or more, and for this group the distribution is as follows:

Duration in years	2	3	4	5	6	7	8	10	11	15	Several	Many	Since puberty
Number of cases	14	9	8	4	5	3	1	1	1	2	11	1	2

From these cases the beginning of the enlargement may be roughly estimated. Among those who gave a history of 2 years' duration was a boy of 14 and one of 17 years. All the others were in their twenties, except one who was 56, and the age of one was not given. Of those in the 3-year group, all were in their twenties except one who was 18, and the age of one was not recorded. All of those with symptoms of from 4 to 11 years' duration were in their twenties. One whose history went back 15 years was 29, and another 31 years old. Two of those with a history of "several years" were 30, all the others were in their twenties, except one whose age was not given. One who claimed to have had the lesion "since puberty" was 22, the other 30. The man who said "many years" was 22! The earliest age of onset claimed was 12 years, but in several others it was 13 and 14. A wide variation in stated age of onset is evident.

Duration in cases of bilateral involvement was recorded in only 6, and in 5 of these covered a period of years. A man, 52 years old, claimed the swelling had been present for only 3 months. The relation of duration of the bilateral condition to age is shown in the following table:

Duration in years	2	3	4	5	6	11
Age of patients	20	Not given	25, 20	20	21	23

The Chinese, 27 years old, had unilateral enlargement which had been present 2 years. The Indian, 21 years old, had had unilateral

involvement for "several years." The Puerto Rican, 50 years old, had had his unilateral lesion for 3 months.

Rate of Progress. The account of rate of progress of gynecomastia is confusing in the 65 histories in which it is recorded, probably because of misuse of relative terms. The growth is said to have been "slow" in 23 cases, yet the time for this slow growth varies from 2 and 3 months to 6 years. It is said to have been "very slow" in two instances, in one of which the duration was 2 weeks and the other 2 years. Growth was "gradual" in 20 instances, but the time was given as from 5 weeks to "several" years. "Rapid" was applied to 10 cases, the duration being from 1 month to 6 months. "Moderate" was used for 3 cases of from 6 weeks to 6 months. The growth was "progressive" in 3 cases, with durations of from 1 month to 4 years. Two patients, with disease lasting 1 year and 7 years respectively, had "recurrent swelling." One patient had had the swelling for 11 years with rapid growth in the last 4 months; another had had the lesion for 6 years, with rapid increase in size for 1 month.

In spite of the unreliability of histories and interpretations, it is evident that there was progressive enlargement continuing from a few weeks to several years. In only 4 cases was there noticeable periodic alteration of rate of growth.

Relation of Trauma to Onset. A history of injury was given in 28 cases and denied in 1. A "blow" received from 3 weeks to 3 years previously was recorded in 11 of the 28 cases. Injury, without specification of its nature, was reported in 8 cases, with a duration of from 4 months to 8 years. Minor injury had been sustained by 1 patient, 4 months before operation. "Possible injury" appeared on 1 record, 2 years earlier. Two patients told a story of "probable injury," each 3 months earlier, and another with a 3-year history had been "bruised" 3 months before operation. One patient struck a board 2 years before, and another was struck in wrestling 3 years before. One patient said the breast was irritated by a gas mask strap 3 months previous to operation. Another, whose lesion appeared 15 years after an injury, said that he sustained a second injury 2 months before operation.

Subjective Symptoms. Pain was a complaint in 50 cases in which duration varied from 2 weeks to several years. Pain was qualified as sharp in 1 case (3 months), slight or mild in 4 cases (1, 2, 2, and 3 months), moderate in 1 (10 months), and persistent in 1 (4 months). Tenderness was a symptom in 24 cases, in which the duration of the lesion was from weeks to several years. It was occasional in 1 case

of long standing and extreme in 1 case of 4 years. Pain and tenderness were associated in 6 cases in which duration of the lesion varied from 3 weeks to 2 years. Soreness was reported in 3 instances, in 1 of which the lesion had been present 6 weeks, in another 6 months, and in the third 7 years. Soreness and tenderness were associated in 1 case of unknown duration. Sharp pain was the complaint of 1 patient, whose lesion had been present only 2 weeks. The enlarged breast of 1 patient, in whom the swelling had appeared 4 years before, was "painful if struck." Progressive tenderness and pain accompanied a lesion with a duration of 1 month, and "dull ache" another of 2 months. Patients who had had swelling for 3 months, 18 months, 2 years, and 7 years said that they had been troubled by pain for 1 week, 3 weeks, 6 months, and 1 month, respectively. Pain had been present in 1 case for 3 months, swelling for only 2. In another, tenderness had been noted for the last 2 weeks although the lesion had appeared 10 months before. In the majority of cases the duration of the subjective symptoms was not indicated.

The psychic factor, although it is not specifically named in the records, is important and will be dealt with in the discussion.

Weight of Tissue. Most of the weights were determined after arrival of the specimens at the Army Institute of Pathology, and cannot be consistent or representative, since skin and nipple were included in some and only the gland or portions of it in others. For these reasons control weights of normal breasts are of no value. The nodule appeared to weigh from 26 to 100 gm. (11 cases); however, it was less than 25 gm. in 8, between 126 and 175 gm. in 7, and as much as 325 and 369 gm. in 2 cases. In 1 case of bilateral involvement the weights were 135 and 152 gm., whereas in another they were 369 and 25 gm.

Size of Specimen varied so widely that figures are worthless. Most measurements are given in the metric system, but a few are indicated vaguely as the size of pullet's egg or of a walnut.

MICROSCOPIC FEATURES

Sections were stained by the usual hemalum and eosin method, after fixation in formalin. Nearly all tissues were embedded in paraffin; only a few were frozen sections. Controls were breasts of young men, presumably normal, secured at autopsy. These were furnished in part by Lt. Col. William B. Wartman, in charge of laboratories at Dibble General Hospital, and in part were from material collected at the

Institute of Pathology, Western Reserve University. The description of the various alterations in the breast will follow the following outline:

- Proliferation
  - Connective tissues
  - Epithelial tissues
    - Increased number of lining layers
    - Sprouts or buds
    - Lacunae
    - Budding of ducts
  - Mixed connective and epithelial tissue
    - Papillae
    - Intracanalicular growth
- Secretion
- Inflammation
  - Periductal
  - Perivascular
  - Diffuse
- Neoplasia
- Resolution
  - Desquamation
  - Ulceration
  - Healing
- Apocrine glands
- Dermatitis

Proliferation

Connective Tissue. The enlargement of the mammary gland in gynecomastia is due principally to proliferation of connective tissue. Although adipose tissue is present, in true gynecomastia it does not contribute significantly to the size of the breast. The connective tissue mass shades into the surrounding substance of the region without sharp definition or indication of encapsulation.

In gynecomastia, it is possible to distinguish a stroma, usually of dense collagenous tissue, and periductal connective tissue, the former occasionally arranged loosely because of edema. The stroma differs from that of normal controls only in amount. Fibroblasts were encountered in the stroma in several cases, always in association with some degree of what appeared to be inflammation. Thus, although fibroblastic multiplication must be responsible for augmenting the connective tissue, mitotic figures are rarely seen, and then may possibly be a sequel of inflammation.

Periductal connective tissue is often loosely arranged, but not because of edema since there is no precipitate in the spaces between fibrils. It is a structural peculiarity without definable cause, which is not constant in gynecomastia, nor even uniform in all parts of the same section. Indeed, it may occur on one side of a duct and not on the other. Fibroblasts, often with amphophilic or slightly basophilic cytoplasm, how-

ever, are always present in this loose tissue. Here, too, are mononuclear cells (lymphocytes, plasma cells, and large mononuclear cells). Thus, although the tissue may be loose because of active proliferation, an inflammatory component cannot be excluded. The presence of mucoid degeneration in this region will be taken up later. Mitotic figures were not found in the connective tissues in this series.

The presumably normal controls lacked such a clear distinction between loose periductal tissue and dense stroma, although suggestive pictures were encountered.

Epithelial. In addition to proliferation of the connective tissue, the ducts are increased in number (Fig. 4) but this varies from specimen to specimen and in different parts of the same section. Ducts may be considerably elongated and with varying degrees of distention and branching (Fig. 3). The ducts in the sections were not actually counted but the cases were divided by rough approximation as containing "many," "moderately numerous," or "few" ducts. In the first group there were 78 cases, in the second, 152, and in the third, 47. Even in most of the cases in the last group, the number of ducts appeared to be slightly greater than in the normal controls. Therefore, in at least 250 cases (88 per cent) there was an appreciable increase in the number of ducts.

Mitotic figures, observed in 37 of the 284 specimens, without exception had normal configuration. They occurred in basal, intermediate, and luminal layers of the epithelium (Figs. 5, 6, and 7). In 2 cases of unilateral gynecomastia, the number of mitotic figures appeared to be in excess of that observed in 2 cases associated with choriocarcinoma of the testis, although it was no greater than after administration of stilbestrol in 1 case. Mitotic figures were not found in any of the controls. No sign of nuclear budding was seen. In accord with most modern opinion, amitotic cell division has not been proved. The multiplication of epithelial cells in gynecomastia is the result of mitotic division.

Epithelial proliferation led to an increase in the number of layers in the ducts until there were often five or six. This process was not uniform in all parts of the same duct and the border of the lumen was often irregular in outline. In addition, delicate masses of epithelium, like papillae but without connective tissue stalks, often projected into the lumen (Fig. 8). In 66 cases these epithelial sprouts were not observed. In 58 of these the number of layers in the ducts did not exceed three; in the remaining 8 there were four or five layers. Initiation of sprout formation was probably not due to distention since the two processes were not regularly associated. The principal factor appeared to be the multiplication of the lining cells, which were usually

cuboidal whether or not there was sprout formation. Slight elongation of these cells was observed, but in only few instances were they cylindrical (Fig. 9).

Frequently the epithelial sprouts fused to form tiny locules in the lumina of the ducts (Figs. 10 and 11), but no material of any kind was found in the locules. Occasionally the proliferated cells filled the entire lumen (Fig. 12). In the gynecomastia of choriocarcinoma and in that which followed administration of stilbestrol, no papillae or locules were observed, nor were ducts entirely filled with cells. The multiplication in these cases led to the formation of five or six layers, but this was seen also in some of the unilateral cases. In the normal controls there were two or three lining layers, but no papillae or locules. Multiplication beyond three layers of cells, epithelial sprouts, and occluded ducts were not observed in the controls, which leads to the conclusion that sprouts, locules, and ducts filled with epithelial cells are abnormal.

In only a few sections was there budding of the ducts, either in the form of narrow prolongations, usually branched, with rounded ends, or of small spherical buds projecting laterally from the walls of the ducts or of their branched prolongations. Small spherical buds occurred in small numbers in the control sections. Nevertheless, in the enlarged male breast the buds, when present, were more numerous than normal. That these small buds may be acini cannot be denied, but since they do not form the grouped acini characteristic of the lobule of the female breast and because they contain multiple layers of epithelium they probably are not acini (Figs. 13, 14, and 15).

Mixed. True intraductal papillae, with cores of vascularized connective tissue, were observed in three instances (Figs. 16, 17, and 18). In none of them was there pleomorphism of epithelial cells or invasion. In one case the pathologist in the field suspected carcinoma, because a duct was filled with cells, most of which were desquamated. The duct affected was dilated and the impression was that the papillary projections were secondary rather than primary. Periductal inflammation was present here as well as around other ducts. In no specimen were more than a few ducts involved, in contrast to the condition in multiple papillomas of the female. Therefore, the papillary structure is regarded as the result of the various features of gynecomastia rather than as being neoplastic.

More or less knob-like masses of connective tissue projected into the ducts in nearly all specimens, deforming the outlines of the ducts (Figs. 19 and 20). In only two specimens was this alteration absent and in these gynecomastia was obviously slight. The ducts of the male breast are often somewhat tortuous; therefore, angular indentations are fairly

common in the sections, as illustrated in Figure 21, and the normal controls may show this configuration. They differ from the more or less blunt intracanalicular growths which may produce an outline resembling that of intracanalicular adenofibroma of the breasts of females (Fig. 22). In none of the specimens of gynecomastia did the deformity of ducts equal that commonly seen in the female, nor did the intracanalicular projections ever show the characteristic whorled pattern. Furthermore, as a rule, cellular infiltration is infrequent and sparse in the intracanalicular adenofibroma; in gynecomastia it is common. Thus, the intracanalicular growth in the male cannot be characterized as a manifestation of adenofibroma.

Secretion

Acidophilic hyaline material was observed within the ducts in 19 cases, acidophilic granular material in 39, and acidophilic material, both hyaline and granular, in 15. Basophilic material, found in 12 cases, was always granular. Granular acidophilic and basophilic material was observed in 3 cases; a combination of acidophilic and basophilic material, both hyaline and granular, was seen in 3 cases (Fig. 23). It is highly probable that these materials represent secretion of a sort, rich in protein in acidophilic, and rich in mucin in basophilic masses. Secretion of this kind was found in 91 of the 284 cases and may be presumed to be fairly common in gynecomastia. Hyaline acidophilic masses, small and not numerous, were found in one presumably normal control. Thus secretion of this sort is not strictly normal, at least quantitatively. Mucin-producing epithelial cells were seldom seen (Fig. 25), and in only one instance was there material which morphologically resembled colostrum (Fig. 24). It was in a duct near the nipple and was made up of a slightly basophilic, granular material containing a few cells, each with a pyknotic nucleus and poorly staining, slightly basophilic, finely granular cytoplasm. No vacuoles indicative of fat dissolved in preparation were identified. Thus it is probable that the material is a mucinous secretion containing desquamated degenerate epithelial cells. The evidence available is against the assumption that it is colostrum.

Distention of ducts was noted in 63 cases with accompanying cystic dilatation in 5 cases. For 1 of these (no. 69458) there was only one block. This contained a cyst 9 mm. in diameter, with four or five lining layers of cuboidal cells, which in places showed parakeratosis and keratosis toward the lumen. Many squamous cells were in the cavity. There was no surrounding inflammation. In another case (no. 75271), with moderate epithelial hyperplasia and moderate inflammation, two sections were from the wall of a cyst, at least 10 mm. in diam-

eter. The lining was of large cuboidal cells with deeply acidophilic cytoplasm, such as are seen in abnormal involution (chronic cystic mastitis) of the female.

Definite elongation of distended ducts was seen in 2 cases. No special cause for obstruction could be found in any of the cases of distention, cyst formation, or elongation. No accumulation of secretions was seen, but in all (except the case in which only the cyst was present) there was epithelial proliferation together with subacute and chronic inflammation of the interstitium.

Squamous metaplasia was observed in the cyst noted above (no. 75271) and also in 2 other cases in ducts not notably distended (Fig. 26). Metaplasia is likewise seen in the diseased gland of the female.

In an occasional duct the cells had undergone transformation to large cuboidal or cylindrical types with notably acidophilic cytoplasm and relatively small, fairly dense, centrally located nuclei (Fig. 27). This is similar to the picture in abnormal involution and other mammary lesions in the female. It differs from the normal apocrine gland in degree of stratification, the central situation of nuclei in the cells, the absence of secretory globules and of rounded cell border toward the lumen.

Red blood corpuscles were found in the ducts in a few cases. Whether this was the result of natural bleeding into the ducts, of trauma of operation, or of spreading during preparation of the tissue cannot be determined. In most cases it was probably due to operation, but in a few instances conglutination of the red cells in the ducts of tissues, which otherwise appear to be well fixed, suggested natural hemorrhage (Fig. 28). A brown discharge from the nipple has been reported by some authors, but was not a feature in any case of this series.

Lymphocytes and other mononuclear cells were occasionally present in the ducts (Fig. 29), probably due to inflammation.

The ducts of the nipple were not often included in the sections, but in the few that were there was some multiplication of lining cells, although not to the degree found in the mammary ducts. Sprouts, locules, or papillae were not observed. Basement membrane was often conspicuous, as is normal, but the periductal tissue was not loosely arranged in this region even when the infiltration of mononuclear cells was conspicuous (Fig. 21).

Inflammation

Inflammation in various degrees was constant in this series of cases. For the most part it was periductal, but not infrequently it involved the denser stroma and in a few instances it was perivascular. More deeply

situated inflammation always accompanied periductal inflammation, but the converse was not true.

Near the ducts of the control specimens there were often a few lymphocytes, as well as an occasional fibroblast or capillary. In cases of gynecomastia the periductal tissue was usually loosely arranged and infiltrated with lymphocytes, plasma cells, which were usually numerous, and those large mononuclear cells often called histiocytes, which varied in numbers and proportions (Figs. 30 and 31). Polymorphonuclear leukocytes were observed in periductal tissues in 36 cases, but never independently of mononuclear cells. They appeared in the more remote stroma in only 4 cases in which they accompanied periductal infiltration. Infiltration of eosinophils was found in 11 cases, and in all but one of these there were also polymorphonuclear neutrophils. The eosinophils were both polymorphonuclear and mononuclear, the former predominating. There was in no case such massive or widespread infiltration of cells as to justify a diagnosis of so-called plasma cell or eosinophilic mastitis.

The periductal mononuclear infiltration often extended to some degree into the more solid stroma, but it was absent in 133 cases. In many instances there were only lymphocytes in the interstitium, but in others there were also plasma cells, large mononuclear cells, and, in 4, polymorphonuclear leukocytes. In some cases the infiltration was perivascular (Fig. 32). When cellular infiltrates occurred in the stroma they were also pronounced in periductal situations.

As a part of the inflammatory reaction, fibroblasts were observed in periductal tissues in 241 cases (85 per cent). This fibroblastic proliferation extended into surrounding stroma in 181, but only occasionally formed a conspicuous part of the picture (Fig. 33).

Capillaries were noted in the periductal tissue in 65 cases (Fig. 34). They often appeared to be newly formed, and with the fibroblasts constituted granulation tissue (Fig. 35). Interfibrillar mucoid material was observed in loose perivascular tissues in 30 cases, but the basic stain of the sections was so often inadequate that this figure is probably low. This material, when present, was highly characteristic (Fig. 56) and certainly occurred more frequently than in comparable lesions in the female.

Exudate was almost always confined to the extraductal part of the breast. In a small number of cases exudation of lymphocytes or of polymorphonuclear leukocytes occurred into the lumina of ducts. In 2 cases the continuity of exudate around and within the ducts caused interruption of the lining epithelial layers (Figs. 36 and 37). Since

exudate was always periductal and only occasionally intraductal, it is probable that extension is into, rather than out of, the ducts.

Although periductal inflammation was associated with ducts of the nipple, exudate was not found within them. In general the involvement of these ducts differed from that of other ducts in the mammary gland. The epithelial proliferation, usually present, was comparable in degree with that in the gland, but there was no indentation by connective tissue growth, only moderate surrounding infiltration by mononuclear cells, no polymorphonuclear leukocytes, and no exudate within the ducts. Although periductal tissues were often conspicuously loose in the mammary gland, a corresponding change had not occurred in the nipple of the same breast.

Neoplasia

True neoplasia was not observed. The intraductal papillary growths have already been described but they could not be regarded as neoplastic. In rare cases, elongated cells filled ducts (Fig. 40), as in intraductal carcinoma in the female, but in no case did this occur in more than a few ducts and in none of these was there the slightest sign of invasion. The section of a lesion of about 10 months' duration (no. 89457), from the right breast of a white man, 27 years old, had in its center a mass of proliferated ducts about 6 mm. in diameter (Fig. 38), which was circumscribed but not encapsulated. The ductules were numerous, varied as to size, were lined with four or five layers of cuboidal cells with occasional mitotic figures, and were surrounded by infiltrated mononuclear cells; capillaries were numerous around the ductules (Fig. 39). There is no justification for considering it an adenoma since it differed from the other lesions of gynecomastia only in its focal character. In this whole series, there was nothing to justify the conclusion that the epithelial hyperplasia or the connective tissue alterations represent true neoplasia.

Resolution

When attempts are made on a purely morphologic basis to demonstrate a sequence of events in, or the processes of, disease, the results may be misleading. Nevertheless, the study of this large series of sections seems to justify tentative conclusions as to one form of retrogression of the lesion.

Desquamation of lining epithelium is frequently observed, but it is difficult to decide whether this is natural or due to fixation and preparation of the sections. However, when the technical work has been excellent it is reasonable to assume that epithelial desquamation, even of considerable degree, occurs naturally in gynecomastia. In only a

few cases did this produce denudation of the wall (Figs. 41 and 42). In 2 cases denudation was associated with local increase of inflammatory reaction. Whether the denudation caused the intensified exudation or resulted from it cannot be determined; however, such local accretions of exudate were not observed without denudation, a fact which indicates that exudation may be the primary event.

Healing of the lesion does not appear to be due to denudation or ulceration, but these processes may sometimes seem to precede resolution. In most instances resolution was brought about by reduction in size and epithelial content of the ducts so that there is a central solid mass of epithelial cells. These cells gradually disappear, leaving the hyaline band of basement membrane, which also disappears as healing advances (Figs. 43, 44, 45, and 46).

Accompanying these alterations in ductal epithelium, there is a notable increase in number of fibroblasts and capillaries (Fig. 35). This increment of what is really granulation tissue is fairly bulky and, as the process advances, there is condensation to a more mature type of white fibrous tissue (Fig. 47). In few cases in this series could the term cicatrix be justly applied nor was there hyalinization of the fibrous tissue.

Some degree of resolution was observed in 41 cases, but in none was the whole lesion entirely involuted. The areas of resolution were generally toward the outer part of a connective tissue mass and adjoining the fat; indeed, many were surrounded by fat. In these cases, other ducts showed epithelial proliferation, sometimes with active mitosis, and periductal or more widespread inflammation. The processes of proliferation and of resolution or involution go on side by side in the same lesion.

The process of involution in gynecomastia is probably not the only means by which the lesion retrogresses. Only by most careful examination of mammary glands which are known to have been previously the seat of gynecomastia can any conclusion be reached.

Fibroblasts, occasionally in great numbers, were often seen in the stroma. However, they were not associated with involution nor did they appear to produce mature connective tissue.

Apocrine and Other Glands

The sudoriferous coil glands, present in the sections, were tightly coiled as are such glands elsewhere (Fig. 48) and in no case appeared involved.

Apocrine glands, just under the musculature in the areolar region, were identified in 37 cases. They were more loosely coiled than the sweat glands and corresponded in detail to the description given by

Schiefferdecker [28] (Fig. 49). The cells were in a single layer, the cytoplasm acidophilic, and the nuclei basal in situation. Myoepithelium was usually seen more readily than in the sweat glands. When the gland was in active secretion, the outline of each cell was rounded toward the lumen and the cytoplasm contained many tiny, intensely acidophilic globules (Fig. 57). In later stages of secretion, acidophilic globules, often fused, appeared in the lumen, the cells were decreased in vertical diameter and the luminal bulge disappeared (Fig. 50). Rarely did the glands under the areola have the irregular outline of those in the axilla. In 8 cases, the apocrine glands were inactive.

No conclusive relationship could be established between the state of the apocrine glands and proliferative or inflammatory change in the associated mammary glands. In the controls the glands were found in both active and inactive states. It is unfortunate that few specimens included nipple and its associated structures.

Although, in cases in which there was dermatitis, infiltration of mononuclear cells sometimes appeared near sebaceous glands, there was no lesion of sebaceous or sudoriferous glands.

Dermatitis

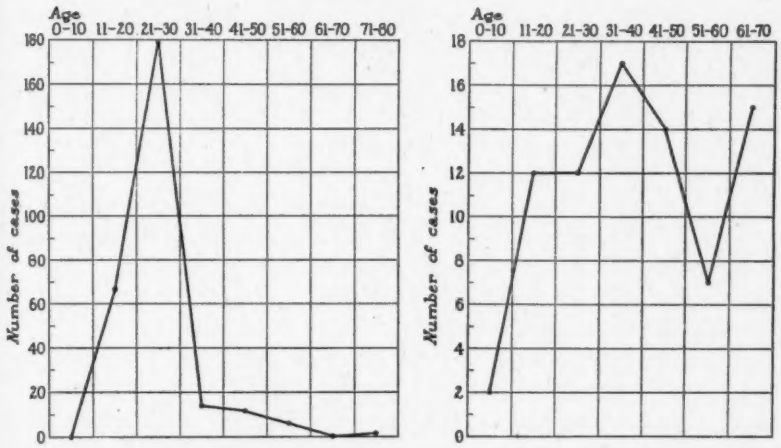
The skin of the normal male breast often contains a few lymphocytes and an occasional fibroblast in the papillary layer of the corium. In 14 cases of gynecomastia, about one-half of those in which skin was included in the specimen, the papillary layer was richly infiltrated with lymphocytes, plasma cells, and large mononuclear cells. The infiltration often extended well down into the reticular layer and was diffuse rather than perivascular or periglandular. In a few cases it was accompanied by dyskeratosis of the epidermis (Fig. 51). In 1 case a distinct crust appeared on the surface, but the section did not disclose actual ulceration (Fig. 52). It is reasonable to suppose that the subacute dermatitis was factitious rather than a part of the gynecomastia.

Discussion

Incidence. The Surgeon General's Office, through Col. E. R. Long, authorizes the statement that in the year 1943 the admission rate to Army hospitals for gynecomastia was 16 per 100,000 personnel. Webster [16] reported that in the Navy the rates for 1939, 1940, 1941, and 1942 were respectively 8.69, 7.40, 9.46, and 6.96 per 100,000. Gross enlargement of the breast is cause for rejection by the Navy; however, men with benign tumor of the breast or chest wall may be inducted by the Army provided the mass does not interfere with the wearing of a uniform or military equipment. In both services, clothing, straps across the chest, and psychic factors are probably about equal in bring-

ing the men to seek treatment. The difference in regulations governing acceptance could account for the divergence in hospital admission rates, although it is impossible to assess the influence of the interpretation of regulations by thousands of local induction centers. Nor is it necessary to assume that the admission rates necessarily represent the actual incidence among the personnel.

Age. Earlier in this paper the number of patients was shown in relation to various decades. The percentile distribution is about 16 in the second decade, 68 in the third, and 16 for the sum of the succeeding decades during which incidence falls rapidly. The general distribution approximates that of Army personnel, but statistical correction is not appropriate because of several factors.



Text-Fig. 1. Age distribution of cases. At the left, the distribution in the present series, set up in accord with Menville's "decades," for comparison with the distribution in his series, as shown at the right.

In a series, reported by Lewis and Geschickter,[24] of 95 cases in a large civilian hospital, the age was usually more than 40 years. The incidence in Menville's [2] 88 cases was highest between 31 and 40. In order to compare the figures in the 284 cases in the Army group with the smaller number in a civilian hospital, our distribution has been recalculated on the same basis as was Menville's. Text-Figure 1 shows the difference in "curves." The larger data show what is probably the more correct age incidence.

In 1868, Gruber [20] drew attention to the inconvenience and discomfort of soldiers with gynecomastia because of straps and tightly fitting uniforms. All 5 patients reported by Sullivan and Munslow [22] complained of aggravation of the lesion by the pack (pack-strap?). If this

applied to the series here reported, there might be an additional factor in determining age distribution.

The number of admissions for psychic reasons would be hard to establish. In a personal interview, an Air Forces surgeon told of a patient who insisted on operation because of ridicule in the barracks. It is probable that young men are especially subject to psychic trauma, but this cannot be verified, nor is it possible to say how many operations were performed on this account. Parachute straps do not irritate the breasts and the number of operations for gynecomastia in the Air Force is small, facts which may be used as evidence that the psychic factor is not important. Webster [16] indicated that all factors enumerated enter into operations on Navy personnel.

Gynecomastia has been reported in more advanced life; 2 patients in this series were respectively 76 and 77 years old. It has been observed in prepuberal life; for example, Ingleby [27] reported cases of boys 7 and 9 years old in which the lesion was unilateral and there was no indication of endocrine disturbance. It may be said that although gynecomastia may be observed at almost any time of life, it occurs most frequently in young men.

Race. Deaver and McFarland [11] intimated that gynecomastia is especially prevalent in African tribes, but the evidence is tenuous and based largely on the reports of lay travellers and explorers. Neither in other publications nor in this series is there any indication that gynecomastia is predominant in any one race.

Breast Involved. Although earlier writers and even an occasional recent one [30] stated that gynecomastia is usually bilateral, Menville [2] reported bilateral disease in only 12.8 per cent of 88 cases and Lewis and Geschickter [24] said it is most often unilateral. Geschickter [20] found it to be bilateral in 20 per cent of 108 cases. In this series, which is larger than either, bilateral involvement occurred in only 12 cases, or 4.6 per cent of the 262 in which location is given. Inequality was noted in only 1 case. Twice, bilateral lesions accompanied choriocarcinoma of the testis with metastases. Once they occurred in a patient with tuberculosis of the adrenals and Addison's disease which had been undiagnosed and untreated. One patient, 52 years old, with bilateral gynecomastia had been operated on for carcinoma of the bladder, but there is no note on the condition of the testes and there was no autopsy. There might have been testicular atrophy. The other 8 patients had no evidences of endocrine abnormality.

The figures in this or any other series may not be fully representative. Slight or even moderate equal enlargement of both breasts may not be noticed by the patient or the examiner, whereas enlargement of only one breast is likely to be obvious to both.

In the few instances of bilateral involvement, in which separate specimens were submitted from the two breasts, the lesions were microscopically identical. Thus, whatever causative factor or factors operated, the effect was the same in each breast.

Duration of Complaint. It may be said that in some instances the lesion appeared at or shortly after puberty, although in most it began in later life.

Jung and Shafton [31] made 1000 clinical examinations on 700 males between the ages of 9 and 45 years. They coined the term "subareolar node" to indicate a firm, disk-like mass under nipple and areola which they concluded appeared as an "integral part of the process of puberty." Even so, the nodes might be asymmetrical, were frequently tender and remained so for months. The involution was highly variable both as to promptness and completeness. In their series, 3 boys between 14 and 18 years had evident gynecomastia (without any signs of endocrine abnormality) in contrast to youths 17 years old with no remaining vestige of the node. Only 12 of 84 men in the 20.5 year group still had palpable nodules in one or both breasts.

It is evident that gynecomastia may be a continuation or augmentation of the pubescent node. It is equally evident that the lesion may arise in later life when the node can be assumed to have disappeared. There is no concrete evidence that gynecomastia is necessarily the sequel of the pubescent state. Adair [30] suggested that it may appear in the "male climacteric," but even if that state actually exists, there would be difficulty in finding support for the statement.

It is said that once gynecomastia develops it is permanent (Word and Reed,[9] Charache [32]) but the present study throws no light on the validity of that statement.

Rate of Progress. Judging from the 65 histories in which significant statements are recorded, progressive enlargement proceeded from a few weeks to several years, but most usually for a few months. Recurrent swelling was reported in two lesions lasting respectively 1 and 7 years. One enlargement present 11 years (no. 82515) grew rapidly in the last 4 months; another of 6 years' duration (no. 92295), in the last month. In case 82515 the number of ducts was markedly increased, whereas in case 92295 the increase was moderate. In both there was distinct epithelial proliferation, but not more than in other breasts that had been enlarged for years, and there was also moderate infiltration of mononuclear cells in the loose periductal tissue. Mitotic figures were observed in case 82515, but not in case 92295. The former also had a greater number of fibroblasts in the dense stroma. There was nothing seen in the microscopic examination which would separate these two

lesions clearly from others which lacked terminal acceleration of growth.

Trauma. The history of injury of some sort in 28 cases and of "possible injury" in 2 others is so vague, in general, as to be practically worthless. Various questions remain unanswered. Did gynecomastia exist before injury? Did injury accelerate the rate of growth? Did it simply attract attention to the lesion? Was it of enough magnitude to be significant? Did it act to provide receptors for endocrine stimuli? Examination of the sections gives no answer to these questions.

Various authors have referred to the effect of trauma, including Erdheim,[3] Word and Reed,[9] Webster,[16] Gill,[18] and Sullivan and Munslow.[22] The type of injury is highly variable. These writers made no important comments as to the relationship. Cheatle and Cutler [5] viewed with doubt the validity of injury as a cause. Impressive is the fact that neither in this series nor in the literature is there mention of injury in instances where outspoken endocrine disturbance appears to be causative.

Subjective Symptoms. Tenderness, dull ache, and pain of various intensities are frequent subjective symptoms. Erdheim [3] characterized the pain as "drawing" and as "lancinating." The fact that 94 patients complained of subjective symptoms suggests that they may be an integral part of the clinical picture. They seem unrelated to trauma, for only 2 of the patients who spoke of tenderness or pain gave a history of injury. In only a few did the swelling antedate the pain; on the other hand, in 1 case the pain was said to have preceded the swelling. Pain in the breast has been reported but is infrequent in gynecomastia associated with choriocarcinoma or following administration of stilbestrol; the more common complaint is a sense of fullness in the breast.

Sections from tender and painful breasts were reviewed especially with reference to inflammation and involvement of nerves. These did not differ in any essential from sections from breasts that gave no subjective symptoms. In neither group was there inflammatory or other involvement of nerves.

Microscopic Features

The multiplication of ducts, the proliferation of epithelium, the formation of papillae and of intracanalicular masses of connective tissue observed in this study have also been described by others. There is general agreement among critical writers that acini and lobules are not formed. However, there is some difference of opinion and this confusion might be due in part to interpreting objective observations to fit the theory that gynecomastia is due to the action of substances with

the effects of estrogen and progesterone. Authors often attach too little importance to the alterations in the stroma. Ductal multiplication and epithelial proliferation may produce changes in particular parts of the gland which are like those observed in abnormal involution (chronic cystic mastitis) and its variations, but the lesion as a whole does not correspond to, nor is it in any way identical with, adenofibroma of the female. In fact, one case of adenofibroma of the female was included with the group of sections but it was readily distinguishable. To be sure, the individual changes in ducts can be found in various conditions in the female breast, with and without cancer, as described by Foote and Stewart,[33] but the association of the various ductal lesions is fairly characteristic. Furthermore, the picture is completed by the changes in periductal tissues and stroma as well as the infiltration of mononuclear cells. Even allowing wide variations, the over-all appearance differs significantly from that of other mammary diseases.

Secretion

It is said casually by some writers that milk may be secreted in cases of gynecomastia. There are only a few references, however, in the literature to actual secretion of a fluid spontaneously or by expression. Zum Busch [21] reported that a "milky fluid" could be expressed in his case of gynecomastia associated with adrenal cortical tumor; Lisser [34] reported a watery fluid in similar circumstances. Secretion of a watery clear, a yellow, or a mucinous material in 3 of the 12 cases of gynecomastia reported by Erdheim,[3] was spontaneous in 2. Geschickter [20] found a discharge from the nipple in only 2 of his 108 cases. Del Castillo, de la Balze, and Reforzo Membrives [17] expressed a fatty liquid from enlarged breasts of a man with carcinoma of the lung, diagnosed as such only by roentgen study. Starr [35] said that in Basedow's case of gynecomastia in hyperthyroidism the swollen breasts secreted colostrum. John Hunter,[36] about 1790, referred to a man who suckled 8 children, but there is no satisfactory evidence that the man had gynecomastia nor that he secreted milk; he was not a hermaphrodite. Gynecomastia was not reported in Hänel's [37] case. Numerous authors have referred to the case of the Zulu chief, Chenwayo, whose name is variously spelled. This man was about 55 years old, weighed about 400 pounds, had 40 wives and, as the story has grown, he nursed more and more children. As a matter of fact, the original report is based on a picture exhibited by a lay travel-lecturer. The statement by Major Shufeldt [38] is: "It is stated that he is the father of more than one hundred children, and the supposition is that he had nursed some of them." This is hardly acceptable evidence.

Of interest was the observation of Roth [39] in a case of acromegaly. The breasts were not enlarged but the nipples projected and the areolae were enlarged. On moderately strong pressure a milky fluid was extruded in which were fat globules but no leukocytes or colostrum corpuscles; it was alkaline and contained 8.5 per cent sodium chloride as well as casein, fats, lipoids, and lactose. There were no data on microscopic examination of the mammary tissue.

There is no satisfactory evidence that the mammary gland of gynecomastia secretes milk. Experimental evidence is suggestive but not conclusive. Van Heuverswyn, Folley, and Gardner [40] found that sex hormones, both male and female, excite a secretory response in spayed female monkeys, but the exact nature of this secretion is not stated. Frazier and Mu [41] administered over a long period the estrogenic substance from the urine of pregnant women to male rabbits. "Milk" could be expressed from the nipples; the fluid from 6 animals became thick and white, from 2 it remained thin and watery. The animals willingly fostered young and 2 suckled them. Three animals, 8 days old, survived 5 to 6 days, 1 to 2 days longer than controls. None of this proves that the material was milk. Brownell, Lockwood, and Hartman [42] treated adrenal cortical extract so as to produce a substance which they called corticolactin. Adrenalectomized mother rats given this material raised 64 and 41 per cent of their young as compared with 2 and 13 per cent raised by those given cortin alone and 72 per cent raised by normal controls. This work has not been confirmed and some investigators regard the evidence that corticolactin is a special hormone as tenuous. Goldzieher [43] suggested that the effects may be due to estrogenic substances in the extract. The appearance of witch's milk is probably due to maternal hormones. No definite evidence exists that milk is produced by these experimental methods.

That secretion occurs in gynecomastia is undoubted. In the 284 cases in this series, albuminous as well as mucinous secretion was noted in the sections; and in the duct of the nipple in 1 case a material like colostrum was found. In no sense can these secretions be thought of as peculiar to the mammary gland as compared with other glands.

Inflammation

Only a few writers have reported the presence of mononuclear or other cells in the loose periductal tissue or in the dense stroma. The fact that polymorphonuclear leukocytes are sometimes associated suggests that such cells are a part of inflammation. Nevertheless, there may be a reversionary factor, to judge from the reports of von Eggeling, Hoepke, and Kolmer [44] that in fetal life hematopoietic centers may occur near tubules and in the connective tissues but disappear by the

time of birth. These centers contain immature erythrocytes, eosinophilic myelocytes, cells with the structure of plasma cells, others of various forms with neutrophilic or basophilic granules in the cytoplasm, giant cells and histiocytes, all probably formed from adventitial cells of the blood vessels. However, the cells in gynecomastia are like those seen in interstitial inflammation in other organs where there are no pre-existent hematopoietic centers. The subsequent development of granulation tissue favors the view that the cells are exudative and the process inflammatory.

Similar infiltration into the stroma may be perivascular. In this situation granulation tissue was not observed, but fibroblasts were fairly common.

Neoplasia

In the material studied there was no true neoplasia, either benign or malignant; neither was there clear evidence in the literature that gynecomastia may develop into cancer. Retraction of the nipple may occur, especially when cysts are present or when concurrent inflammation has caused adherence to surrounding structures. Occasionally confusion may result from enlargement of the axillary lymph nodes; however, Erdheim [8] has pointed out that this process is only simple hyperplasia.

Word and Reed [9] stated that "carcinoma and sarcoma have both been observed to develop subsequent to gynecomastia and fibroma of the male breast." Gilbert [45] said that 9 (19 per cent) of his patients with carcinoma of the male breast had gynecomastia. Cheatle and Cutler [5] reported 1 case of gynecomastia with malignant papilloma. Geschickter,[20] however, found no malignant disease in 108 cases of gynecomastia. Ewing [14] stressed the importance of chronic cystic mastitis as a precursor of carcinoma of the breast in the female, but did not suggest that gynecomastia leads to cancer.

The reports of malignant changes in gynecomastia are evidently based on the assumption that any enlargement of the male breast is gynecomastia. No consideration is given to the possibility that enlargement may be due to the neoplasm rather than to the phenomena which constitute gynecomastia. The fact that Geschickter [20] noted no neoplastic disease in his series and that none was found in this larger one must indicate that neoplasia, if it ever follows gynecomastia, is exceedingly infrequent.

Resolution

There is a general impression among clinicians that once gynecomastia is established it does not naturally undergo retrogression. The material studied in this report gives no evidence one way or the other, but the assumption of others is probably valid. Nevertheless, focal

retrogression or resolution does occur, but there is no ground for stating that it affects the mammary gland as a whole. Indeed, the fact that such resolution is concurrent with active progress of the disease leads to the contrary view. Such foci of resolution are rarely seen in other lesions of the breast, except, as described by Muir,[46] in the intraductal carcinoma of the mammary gland associated with Paget's disease of the nipple.

Apocrine Glands

The apocrine glands may be active or inactive quite independently of the proliferative or inflammatory condition of the associated mammary gland. If endocrine activity plays a part in gynecomastia, it is impossible to correlate it with the apocrine glands.

"Apocrine epithelial proliferation" (so-called pale glands) is seldom observed deep in the mammary gland in gynecomastia. In the material reported herein, pale glands were not large enough to be visible grossly, as described by Foote and Stewart [33] in various diseases of the female. The acinic structure is different from that of true apocrine glands. Although Ewing [14] suggested that apocrine glands are derived from the sudoriferous coil glands said to accompany mammary ducts, it seems more probable that they represent a change in proliferated ductal epithelium, which may be metaplasia or a reversionary process to be explained by the embryonal origin of mammary and sudoriferous epithelium. At any rate, these altered ducts differ in situation and structure from the subareolar apocrine glands.

Dermatitis

Subacute dermatitis, sometimes with ulceration and crusting, is fairly frequent. It is not an essential part of gynecomastia, but may be due to irritation or may possibly be factitious. Ewing [14] spoke of chronic eczema as a contributing factor to mammary carcinoma of males, but the lesion seen in this series would not qualify as eczema.

Etiology

It is probably true that, as Erdheim [3] said, in most cases gynecomastia is the primary disease; in some, however, the lesion is obviously secondary to other conditions, and in still others presumptive causes are mentioned without proof that they are effective.

Heredity has been suggested as a factor. Savitsky [47] reported a patient, 21 years old, with massive pendulous breasts, whose father, a brother, and a cousin also had large breasts. The exact nature of the lesion was not investigated. Hutchinson's [48] case of the son of an acromegalic woman was certainly pseudogynecomastia. I agree with

Cheatle and Cutler [5] that there are no satisfactory records of hereditary influence. Among the cases of gynecomastia so far reported there is nothing to indicate a hereditary or constitutional affection of endocrine glands.

Endocrine Factors

Testes. In 1894, Williams [15] attributed gynecomastia to various diseases of the testis, to atrophy, to injury, and to excision of these gonads. According to Menville,[2] Schmit had reported the lesion associated with orchitis in 1891, and Martel with varicocele in 1893. Subsequently, Weber [4] asserted that gynecomastia might be the sign in adults of destruction of the testes, and attributed gynecomastia to lesions of the testis except those in which the interstitial cells are spared. Tellgmann's [49] widely quoted case of gynecomastia on the same side as a testis which had been crushed and destroyed was almost certainly pseudogynecomastia, especially as he stated specifically that glandular tissue could not be palpated. Cheatle and Cutler [5] gave credence to the view that disease and atrophy of the testis are followed by gynecomastia. Ilabaca Leon [8] associated the lesion with various genital defects. Webster [16] said that in a few castrated males and in some with prostatic enlargement, gynecomastia was associated with endocrine imbalance. Richardson [50] suggested that testicular atrophy may cause reduction in amount of male sex hormone.

In none of these reports is concrete evidence offered on the basis of actual assays of endocrine secretions.* Furthermore, in many instances no distinction has been made between the fatty breasts of pseudogynecomastia and true gynecomastia. Pratt [51] pointed out that the breasts may share in obesity following bilateral orchiectomy in prepuberal life and occasionally in adults. Zondek [52] said that eunuchs may have fatty breasts. Lewin [23] stated flatly that bilateral excision of the testes does not produce gynecomastia. Kriss [1] maintained that neither in man nor in animals is there anything to support the view that the testes influence the development of the breast. Erdheim [3] excluded nonneoplastic testicular lesions as causative of gynecomastia.

Nevertheless, there is experimental evidence of some relation between mammary gland and testes. Richardson [50] reported that castration prevents the normal development of the breast in male rats at puberty. Deanesly and Parkes [53] showed that certain androgens may produce some of the manifestations of feminization but did not refer

* Throughout the following discussion, emphasis is laid on the importance of assays of hormones or their products. This is with full recognition of the sources of error and the fact that such assays do not necessarily indicate what happens to the hormones in the body. Yet the assays are the nearest objective approach to the problem.

specifically to gynecomastia. Selye, McEuen, and Collip [54] described slight development of the mammary glands in gonadectomized female rats when testosterone benzoate was administered. Van Heuverswyn, Folley, and Gardner [40] reported that androgens induce growth of the main ducts of the mammary glands of male guinea-pigs, of immature male and female rats, and of spayed virgin rats, but that the effect of different androgens varies. There is also a body of evidence to indicate that gynecomastia may be due to estrogens. If that be so, another effect of estrogenic substances is atrophy of the testes. Dunn [55] reported atrophy of the testes in "hypersexual" males following administration of stilbestrol, but testicular tissue was not examined microscopically. McEuen, Selye, and Collip [56] showed, however, that the mammary gland does not develop in male rats castrated in early life and, further, that castration of adult males leads to involution of the already developed breast. It is well known that in Cushing's disease and various tumors of the endocrine glands the testes become atrophic, usually as a result of deterioration of the seminiferous tubules while the interstitial cells may be reduced, increased, or of about the normal number. Frazier and Mu [41] found that administration of pregnancy urine to rabbits led to atrophy of the testes, but this had no microscopic confirmation. Bonser and Robson [57] found that the effects of prolonged administration of estrogens differed for three strains of mice. In CBA and R III strains, the testes became atrophic and spermatogenesis ceased, but in the Strong A strain, spermatogenesis persisted in spite of marked hyperplasia of interstitial cells. These and other reports support the view that estrogens may produce testicular atrophy, but their effects are not uniform.

The relation of testicular disease, other than neoplasia, to gynecomastia is not clear. Certainly in man there are too many cases in which the testes are morphologically and functionally normal to justify the belief that atrophy or destruction of the testes is a significant cause of gynecomastia.

Tumors of the Testis. The tumors of the testis concerned in gynecomastia fall into two great groups: (1) teratoma and choriocarcinoma, and (2) interstitial cell tumors.

Lewin [23] quoted Heidrich as reporting gynecomastia in 8 of 140 patients with teratoma testis, and Ferguson in 5 among 117. It has been difficult to determine how many of these are cases of choriocarcinoma, how many of embryonal carcinoma, and so on through the list of testicular tumors. Kriss [1] considered testicular tumors the most frequent neoplastic causes of gynecomastia, but this is not proved. In my Army series testicular tumors found were 2 embryonal carcinomas,

4 malignant teratomas, and 1 choriocarcinoma. In some of the cases in which teratoma was diagnosed, the tumor is undoubtedly choriocarcinoma. An example of the confusion of nomenclature is the report by Craver and Stewart [58] of a choriocarcinoma under the name teratoma, undoubtedly due to Ewing's claim that choriocarcinoma and embryonal carcinoma represent one-sided development of a teratoma.

Cairns [59] observed gynecomastia in 2 of his cases of neoplasm of the testis identified as teratoma, and found that the enlargement of the breast coincided with the development of metastases. He cited cases of Cooke, of Warthin, and of Malloch, all of which were choriocarcinoma; also a case of Garbarini, in which the gynecomastia subsided after removal of the tumor. Bonn and Evans [25] reported a case of extragenital choriocarcinoma in which one breast became enlarged before the other. In this case the Friedman test was positive and the assay for prolan showed more than 10,000 and less than 100,000 units. Perhaps more instructive are the assays in the case of extragenital choriocarcinoma with bilateral gynecomastia reported by Laipply and Shipley,[60] as follows:

Hormone Assays on 24-Hour Urine Specimens

Case of Laipply	Gonadotrophin Chorionic Type in Mouse Units	Estrogen International Units	17-Ketosteroids in mg.	Pregnanediol in mg.
and Shipley	300	750+	27	2+
Normal male	0	75—	25	0.1
Normal female	0	50–300 (ovulation)	12	45 in luteal phase
Teratoma testis	Elevated	Not known	No change	Not known
Late pregnancy	6,000	10,000	No change	30–40
Choriocarcinoma of uterus	200,000 to 1,000,000	Elevated	No change	Not known

The increase of chorionic gonadotrophin,* of estrogen, and of pregnanediol is apparent, but there is no significant change in 17-ketosteroids. No particular influence of gonadotrophins on the breast is known, but in certain animals estrogens stimulate proliferation of ducts, and progesterone, when acting with estrogens, the acini. In this case ductal proliferation was marked. The slight increase in pregnanediol, as an index of progesterone, did not lead to acinus formation, but the level may have been too low for such an effect to be expected. In Bonn and Evans' [25] case, the high prolan may have operated through the medium of other factors. In Craver and Stewart's [58] case with high content of follicle-stimulating hormone, gynecomastia is not mentioned.

Bilateral gynecomastia occurs in cases of tumors of the testis of the

* The spelling now widely used by endocrinologists has been adopted in this paper.

general order of choriocarcinoma and probably in other tumors, presumably because of increase in estrogenic substances. As yet there is no clear indication that progesterone is effective nor can gonadotrophins be directly incriminated, especially since in Laipply and Shipley's [60] case the increase was in chorionic gonadotrophin rather than that produced by the pituitary body.

Gilbert [61] analyzed 123 cases of what he called the syndrome of choriogenic gynecomastia. These included cases of choriogenic hyperplasia, primary testicular choriocarcinoma, extragenital choriocarcinoma, apparently misdiagnosed testicular tumors, and teratoid tumors of the testis with choriocarcinomatous metastases. Although endocrine assays were made, usually incomplete, in 46 cases, he concluded that "choriongonadotrophic hormones and folliculin exert a stimulating effect on the pituitary, producing pseudopregnancy changes with hyperplasia of the breast and accessory sexual organs." Shimkin [62] pointed out, however, that, at least in mice, chorionic gonadotrophin does not affect the mammary gland.

The relationships of the interstitial cells are not clear. The association of atrophy of the testes with gynecomastia is not frequent and little information is gained without microscopic examination. Choriocarcinoma may be accompanied by atrophy of the testes. In Craver and Stewart's [58] case the seminiferous tubules were atrophic and the interstitial cells hyperplastic, as has been true of my cases. They quoted the cases of de Vries and of den Hartog in which, however, there was no hyperplasia of interstitial cells. Richardson [50] suggested that testicular atrophy leads to absolute reduction in amount of male sex hormone and that the imbalance with the relatively large ratio of estrogens results in stimulation of the mammary glands. The possibility that balance may be preserved by production of androgens in the adrenal cortex is given no consideration, and neither is the demonstration by Van Heuverswyn, Folley, and Gardner [40] that androgens may of themselves stimulate mammary growth. In this connection Klinefelter, Reifenstein, and Albright [63] reported cases of gynecomastia, aspermatogenesis without a-leydigism and increased excretion of follicle-stimulating hormone. They concluded that the gynecomastia in these cases was not due to hyperestrinism nor to androgen alone, but possibly to a combination of androgen and lack of inhibin. Heller and Nelson [64] agreed that increased amounts of gonadotrophic hormone, and low normal or reduced quantities of androgen and estrogen might bring about gynecomastia, but pointed out that there is no proof of the existence of inhibin. Furthermore, they found that gynecomastia does not always occur in the syndrome.

Hunt and Budd [65] reported the case of a white man, 42 years old, with enlarged, painful breasts, impotence, and an interstitial cell tumor of the testis. After removal of the tumor the enlargement of the breasts receded and libido was restored. The urine contained 1000 units of luteinizing hormone per liter, but other determinations were not reported. The cells of the tumor contained mitochondria-like granules, but the crystalloids of Reinke* were not found. This case raises the question common to all tumors of the testis designated as interstitial cell, namely, whether they are derivatives of interstitial cells or of adrenal cortical inclusions. The fact that the interstitial cells normally contain refractile globules of lipids, brown pigment, and the crystalloids of Reinke does not mean that these would occur in neoplastic cells, but if they are found there would be no reasonable doubt of the cytogenesis. In their absence, however, the cells cannot positively be distinguished from neoplastic cells derived from adrenal cortex. Endocrine studies have not been conducted to a sufficient extent to permit of conclusions. In Craver and Stewart's [58] case of obvious interstitial cell hyperplasia without gynecomastia, follicle-stimulating hormone was present in excess, whereas in Hunt and Budd's [65] case the increase was in luteinizing hormone. From the functional point of view the tumor reported by Hunt and Budd might possibly have been an adrenal cortical neoplasm.

Monaschkin's [66] case of testicular tumor with gynecomastia is repeatedly cited as an example of interstitial cell tumor, but it may well have been an embryonal carcinoma. He cited Benda as having offered the speculation that the tumor was derived from embryonal cells of feminine type (origin not further specified) which favor the growth of mammary glands and lead to testicular atrophy. There is little doubt that in certain strains of animals the prolonged administration of estrogens may lead to the development of testicular lesions which have many of the features of interstitial cell tumors, as illustrated in the work of Bonser and Robson.[57] Bonser and Hawksley [67] compared the lesions in 2 human cases with the experimental material from mice and concluded that they were identical, but too little analysis of endocrines was done to confirm this opinion. The Aschheim-Zondek test, performed in only 1 case, was negative.

The influence of interstitial cells of the testis upon gynecomastia and the converse is far from clear. It cannot be proved that interstitial cell tumors are accompanied by gynecomastia until a positive means is

* Reinke, F. Beiträge zur Histologie des Menschen. Ueber Krystalloidbildungen in den interstitiellen Zellen des menschlichen Hodens. *Arch. f. mikr. Anat.*, 1896, 47, 34–44.

established to differentiate in man between tumors of that order and those which may be derived from adrenal cortical rests.

Ovary. Large breasts may be observed in hermaphrodites with ovary and ovotestis. Although these persons may have the secondary sexual characters of males, they are examples of true intersex and the large breasts are attributable to ovarian function. This is not gynecomastia. In cases of intersex the adrenal cortex may be notably hyperplastic. Shimkin [62] reviewed fully the work of Loeb and Lathrop concerning the influence of ovaries on growth of the mammary gland and the development of tumors, but this has no direct bearing except as concerns the effects of estrogen.

Adrenal Cortex. The hormones of the adrenal cortex are known to influence secondary sexual characters. Enlargement of the breasts in males with Cushing's disease or syndrome is such that, as Glass and Bergman [68] said, "gynecomastia may now be included in the category of adreno-genital syndrome." They reported that in virilized females, the ratio of androgen to estrogen was increased but that in 2 men with gynecomastia, the opposite was true. Although Parkes [69] favored the importance of ratio, Goldzieher [43] seemed doubtful.

Several cases of cortical tumor of the adrenal with gynecomastia have been recorded. Bittorf's [70] case of a man, 26 years old, is somewhat dubious because of an inadequate report on the tumor. Mathias' [71] case of enlarged breast in a female, 3 years old, with adrenal cortical tumor is suggestive, but the enlargement was not gynecomastia even though the girl showed many signs of virilism. The patient of zum Busch [21] with painless gynecomastia and secretion of a milky fluid was a man, 27 years old, who had carcinoma of the left adrenal cortex and metastases. Weber [4] included this case in one of his papers. Holl [72] reported 2 cases. One of bilateral gynecomastia and tumor in the region of the left adrenal, which Beitzke called "hypernephroid," was that of a boy aged 15. The other of bilateral gynecomastia and atrophy of the testis was of a man, 44 years old; removal of a tumor from the cortex of the left adrenal resulted in restoration of sexual function and regression in size of breasts. Simpson and Joll's [73] patient was a man, 34 years old, with bilateral gynecomastia and reduced size of genitalia. After the cortical adrenal tumor was removed it recurred. Assays of hormones at this time gave evidence that estrogens were increased and androgens were only slightly increased, chiefly D $^{3:5}$ androstadiene-17-one. Broster and associates [74] reported the case of a man, 38 years old, with bilateral enlargement of breasts, adrenal cortical carcinoma, and an excess of estrogen in the urine. Lisser's [34] patient was a man, 33 years old, with markedly enlarged breasts, firm but not tender. A

malignant tumor near the kidney was called an adrenal cortical tumor, although, since neither the kidney nor adrenal was involved, it was supposed that the neoplasm was derived from an adrenal rest. Unfortunately there were no endocrine assays. "A watery fluid could be expressed at times from the breast." This was interpreted as lactation!

There is sufficient evidence that hyperplasia of the adrenal cortex as well as cortical tumors, benign or malignant, may be accompanied by gynecomastia. Just what hormone or hormones may operate is not clear, but it is unlikely that neutral 17-ketosteroids in the urine are increased. For example, Warren [75] found normal values for 17-ketosteroids in 1 case of gynecomastia. Zondek [52] referred to the heterosexual function of adrenal cortex, as indicated in virilization of females and rare cases of suggestive feminization of males; exact correlation awaits more assays of hormones. Of interest is Allen's [76] observation that tumors of the adrenal cortex may develop in mice after early ovariectomy and that there is evidence that the tumor secretes estrogen; in susceptible strains of mice mammary carcinoma may arise.

Reference has already been made to experiments in which the breasts of animals have been stimulated with androgenic substances. It is noteworthy that in the work of Van Heuverswyn, Folley, and Gardner,[40] the best growth of the breast followed administration of desoxycorticosterone acetate and androstenediene. The reports on treatment of Addison's disease in man by cortical extracts are also suggestive. Edwards, Shimkin, and Shaver [77] observed that a man of 35 years, with Addison's disease due to cortical atrophy of the adrenals, had enlarged, tender breasts after daily injections of eschatin for 3 months. Treatment was stopped and the breasts regressed only to grow again when treatment was resumed. The same sequence was reported in Lawrence's [78] case, in response to desoxycorticosterone acetate. In Raleigh and Philipsborn's [79] case of a man aged 40 with Addison's disease, the right breast became enlarged after 2 years of treatment with desoxycorticosterone acetate. The unilateral enlargement persisted without involving the opposite breast, an exception to the general rule of bilateral enlargement in unquestionable endocrine cases. It is interesting, but does not bear directly on the problem, that a 30-year-old patient in this series with bilateral gynecomastia had Addison's disease with tuberculosis of the adrenals, but since this was not discovered until autopsy, the patient had had no hormonal treatment of any kind.

Gynecomastia may result from administration of two extracts from the adrenal cortex, eschatin and desoxycorticosterone acetate, but this is rare. The significance of this observation is attested to by reduc-

tion in size of the breasts upon stopping treatment and subsequent enlargement when it is resumed, as well as by the effects of cortical hormones on experimental animals. Whether gynecomastia in cases of cortical tumors is to be explained in the same way is not yet established, but in the cases of Simpson and Joll [73] and of Broster and associates [74] the output of estrogen in the urine was increased. Thus there remains a question as to whether adrenal cortical hormones or estrogens were more significant, or whether hormonal imbalance was important.

Pituitary Body. Although several authors speak of gynecomastia in disease of the pituitary body, there is little in the way of documentation. If Cushing's disease be primary in the pituitary gland, certainly gynecomastia has been observed, but in practically all cases the adrenal cortex is hyperplastic. Roth's [39] case of acromegaly, in which the breasts secreted a milky fluid, was without gynecomastia. Moehlig's [80] patient, 52 years old, had had bilateral enlargement of the breast for 2 years. Autopsy limited to the head disclosed a chromophobe adenoma of the pituitary gland. The man was fat, had adenomatous goiter, a female type of escutcheon, and atrophic testes, but there was no indication of true gynecomastia. There may have been other lesions in the body; certainly there was no proof that gynecomastia was due to the pituitary tumor.

Heinbecker [81] expressed the opinion that in Cushing's disease the lesions of the pituitary gland are not primary, but are due to hypothalamic dysfunction. In a discussion of precocious puberty, Weinberger and Grant [82] formulated the hypothesis that lesions in nerve tracts between hypothalamus and pituitary body interrupt inhibitory stimuli to the latter, thus releasing gonadotrophic hormones. If this were the case, androgenic or estrogenic hormones might be increased in amount. Lactogenic hormones are evidently produced by the pituitary body, but there is no proof that they induce morphologic hyperplasia. Except in Cushing's disease there is no regular association between pituitary lesions and gynecomastia.

Experimental study is handicapped because prolactin is the only pituitary hormone isolated in crystalline form and there is no evidence that it stimulates growth or formation of tumors in the mammary gland.[62] Nevertheless, the hypophysis may play a part, because, at least in mice, hypophysectomy inhibits the proliferation of the mammary gland induced by estrogens.

Thyroid. Starr [35] found in the literature 8 cases with associated hyperthyroidism and gynecomastia, to which he added 2. He referred to Basedow's case in which the enlarged breasts are said to have

secreted colostrum. Starr's first patient had bilateral gynecomastia with increased metabolic rate. For technical reasons only hemithyroidectomy was performed and it was followed by reduction of basal metabolic rate and recession in the size of the breasts. With a subsequent rise in basal metabolic rate the left breast again became enlarged. Only the right breast of the second patient was enlarged. After thyroidectomy, the basal metabolic rate which had been increased, fell to minus 3 and the right breast became normal in size. Starr referred to experiments of Weichert and Boyd, who found that the feeding of thyroid to pregnant rats resulted in greater hypertrophy of the breasts than was true of normal controls. Petersen, Knodt, Ludwick, and Pomeroy [83] said that "the rôle of the thyroid in mammary gland development and lactation is still controversial." The general impression is that thyroid substance is not effective in producing mammary tumors.

The augmented metabolic rate apparently was accompanied by gynecomastia, but without hormone assays other factors cannot be excluded. The experiments cited throw no light on the mechanisms concerned. Thus the effects of endocrines other than the thyroid have not been ascertained. The influence of the thyroid upon the cellular structure of the pituitary gland and the stimulation of lactation by thyroxin are well known, but they have no direct bearing on the hyperplasia of gynecomastia.

Hepatic Cirrhosis

Edmondson, Glass, and Soll [84] have referred to the association of atrophic cirrhosis, ascites, testicular atrophy, and gynecomastia as the Silvestrini-Corda syndrome. They said that 65 cases had been reported in foreign literature but none in English. They reported 8 additional cases, in which gynecomastia developed late in the disease after ascites had occurred. The changes in the breasts were principally proliferative like those due to administration of estrone to animals. They cited Sevringhaus, Israel, and others who have shown that the liver acts normally to destroy estrogen, and cited 2 of their own cases in which excretion of total estrogen in the urine was augmented and 2 others in which free estrogen was increased. Schiller and Pincus [85] reported that in rats, extirpation of lobes of the liver interferes with the conversion of estrone to estriol. Although Cantarow, Paschkis, and Rakoff [86] expressed doubt that the liver inactivates estrogens *in vivo*, the demonstration of excess excretion in the urine of actual cases of cirrhosis makes it seem probable that even the rare case of gynecomastia associated with hepatic cirrhosis may well be due to the pro-

longed action of estrogenic substances. The fact that gynecomastia has not been reported in acute hepatic disease may be because of too short an action or because of the absence of the dietary deficiencies which play a part in development of cirrhosis. The testicular atrophy may be like that which accompanies chronic disease in general or may be due to the effects of estrogen.

Prostatectomy

Cheatle and Cutler [5] said that there are numerous instances of gynecomastia in older men following prostatectomy. Oppenheimer [87] reported 1 case of a man, 64 years old, with bilateral gynecomastia which began 3 months after prostatectomy, and another, 48 years old, with bilateral gynecomastia "several" months after operation. He offered the theory that cicatrization of the ejaculatory duct is, in effect, a Steinach operation. Mann [88] reported the case of a man, 70 years old, who acquired bilateral gynecomastia 2 years after prostatectomy. He cited Kandellon as reporting 2 cases, both in men of 70; in one, gynecomastia appeared in the right breast 1 month after operation and disappeared in a year; in the other, the right breast became enlarged 3 months, and the left 4 months postoperatively. He advanced the theory that the prostate may elaborate an internal secretion, but this is pure speculation.

The reason that gynecomastia appears in old men, according to Cheatle and Cutler,[5] is that they are the people who have had prostatectomy. One of Oppenheimer's [87] patients was 48 years old, which suggests that prostatectomy is the important factor. I have found no record in which the lesion followed transurethral resection. Until endocrine assays are made, no conclusions are possible concerning prostatectomy as a causative factor.

Pulmonary Disease

Del Castillo, de la Balze, and Reforzo Membrives [17] reported 3 cases of bilateral, but unequal, painful enlargement of the breasts. In all, sexual potency was reduced and primary carcinoma of the lung had been diagnosed clinically, although in only 1 was it proved by autopsy. These authors cited Secco's 5 cases of unilateral gynecomastia with lesions in the thorax: 2 were pulmonary abscess; 1 was suppurating hydatid cyst; 1, spontaneous pneumothorax; and 1, bullet wound in the left infraclavicular region. These reports prove nothing, but do suggest that men with gynecomastia are not immune to infection, neoplasm, or injury, and that the converse is equally true.

Estrogenic Hormones

The natural estrogenic substances used experimentally include estrone, estradiol, estriol, equilin, equilinin and their benzoates. The synthetics are particularly diethylstilbestrol and its dipropionate, and also triphenylethylene; these do not contain the steroid nucleus but are highly estrogenic. Estrogens excite development of ducts. In some species acini and lobules are produced, but in others progesterone as well is required for the growth of acini.

Lewis and Geschickter [24] injected Amniotin-Squibb into male monkeys and found that the best results were obtained when small doses were given over a long time. Bilateral enlargement of the breasts occurred and microscopically the changes were like those of human gynecomastia. They reported that castration favored regression of the lesion. Chorionic gonadotrophin from pregnancy urine produced similar but less marked changes, but it is not indicated that estrogens were removed from the extract. Gardner and Van Wagenen [89] reported varying results in different species. Estrogens alone gave rise to full mammary development in guinea-pigs. In rabbits, estrone induced growth of ducts, but glands appeared only when extract of corpus luteum was added. In both male and female mice, prolonged administration of estrogens seemed to lead to formation of localized nodules of alveoli. They cited Allen [76] who, by administration of estrin, had partially restored the atrophic breasts of ovariectomized monkeys, and Turner and Allen who stimulated ductal growth in male monkeys and some alveolar development in females by the same treatment. They referred to Aberle who found that young female monkeys gave no response to theelin, whereas growth of ducts was accelerated in young males. Gardner and Van Wagenen reported that the response to treatment with theelin, theelol, or hydroxyestrin benzoate was essentially the same; in male monkeys each induced proliferation of ducts and development of acini.

That these results are directly applicable to man is open to some question and the same is true of the generalization concerning the action of estrin and progesterone. Dunn [90] reported that when estradiol benzoate was used to treat migraine in males the breasts became enlarged, but he said nothing about ducts and acini.

Diethylstilbestrol, 4′4′-dihydroxy-a-b-diethylstilbene, a synthetic preparation with estrogenic activity, has been studied experimentally and has been used in the treatment of the metastatic lesions of prostatic carcinoma. Smith and Smith [91] found that stilbestrol is 100 times as active as estrone in bringing about release of gonadotrophic factors in

mature male rats. These responses are not identical with those to estrone, because in castrated male rats the administration of progesterone depresses the activity of estrone but not of stilbestrol. Smith [92] reported on the lack of identical action by the two substances. In experimental animals triphenylethylene has much the same effect as stilbestrol.

Nevertheless, stilbestrol produces essentially the same changes in the breast as those which may be attributed to natural estrogens. Dunn [93] reported gynecomastia resulting from the treatment of a "sex criminal" with stilbestrol. Moore, Wattenberg, and Rose [26] gave a fuller account of a case of prostatic carcinoma since it included reports of biopsies of the enlarged and sometimes painful breasts before and after treatment with stilbestrol. The mammary ducts became elongated and early showed budding. The epithelium proliferated, producing additional lining layers of the ducts, even to the point of filling their lumina. There was expansion of the connective tissue stroma, which appeared edematous because of pale staining, and later the fatty tissue increased. The photomicrographs indicated inflammation. The authors seemed to regard the process as a chronic cystic mastitis. There were no endocrine assays.

The same authors [26] stated that there were no indications of cancer, nor was there evidence in the literature that stilbestrol produces cancer of the human breast. Although it is true that this does not occur regularly, the development of mammary cancer after administration of natural estrogens in animals is known to be a possibility. The work of Lacassagne,[94] of Burrows,[95] of Bonser,[96] of Gardner, Smith, Allen, and Strong,[97] and others, indicates that administration of estrone benzoate or of ketohydroxyestrin may be followed by mammary cancer in mice. Transplantation of the cancer is reported by Bonser and by Gardner and his associates. Burrows found several instances of hypertrophy of the mammary epithelium in noncancerous breasts and Bonser reported branching of ducts and distention of lumina in which were albuminous material and epithelial débris; these changes, together with a number of small cysts, were reminiscent of chronic cystic mastitis.

By means of biopsy and post-mortem examination, Eisen [98] studied the process in male rats treated with crystalline estrin (estradiol dipropionate). He confirmed the view that, although not strictly delimited, estrogen stimulates multiplication of ductal epithelium and that, when progesterone is combined, the effect is growth of acini and promotion of secretion. These changes are followed by fibrosis, and he too compared the condition to chronic cystic mastitis. The lesion may progress to sclerosing adenomatosis, a condition which Foote and

Stewart [33] attributed to alterations in acini rather than in ducts; however, acini have not been positively identified in human gynecomastia. In 2 of Eisen's 140 rats, adenocarcinoma developed, but there were no metastases and transplantation was not attempted. Thus there is only microscopic evidence of cancer. The possible fallacies of histologic diagnosis of malignant disease are well known, and confirmation by successful transplantation is desirable. The work on estrogenic hormones has been reviewed by Gardner,[99] by Allen,[76] and by Shimkin,[62] who stressed the importance of certain features. Genetic factors cause variations in response in different species and strains. The carcinogenic action of estrogens is especially effective on organs which are naturally stimulated, and is generally proportional to their physiologic activity, *i.e.*, up to certain limits the action reflects the dosage. In mice, the milk factor is highly significant, but is not the sole determiner of the effects.

Observation justifies the conclusion that in men estrogenic substances provoke the development of gynecomastia, but there is no satisfactory evidence that the lesion becomes cancerous. In male mice and rats, natural and synthetic estrogens induce proliferative and cystic changes with occasional development of carcinoma. It cannot be said, however, that the noncancerous lesion in rodents is the equivalent of gynecomastia in man; rather it resembles chronic cystic mastitis of women.

The enlargement of the breast in gynecomastia is undoubtedly due in large part to the growth of connective tissue. Von Haam and Cappel [100] pointed out conflicting reports in the literature as to the effects of hormones on the multiplication and growth of fibroblasts. In their experiments they used the hearts of mouse embryos to obtain tissue cultures of fibroblasts, and applied theelin, progesterone, and testosterone in crystalline form. In low concentrations estrin produced a slight increase in mitotic activity and a moderate increase of surface area of growth, but higher concentrations were inhibitory. Progesterone and testosterone were about equally inhibitory. Although they concluded that these sex hormones "could not be regarded as significant growth-stimulating agents," the possibility still remains that in low concentration and acting over a considerable period of time, estrogenic substances might be responsible for the connective tissue proliferation in gynecomastia. Although perhaps not directly applicable, the fibromatogenic effects of estrogen and its metahormones are suggestive.[101]

Estrogens and Unilateral Gynecomastia. I have found no reference to hormone assays in unilateral gynecomastia. Can it be said that the lesion in unilateral gynecomastia differs fundamentally from that of

bilateral involvement or that with which hormonal changes are associated? The lesion in one breast is identical to that in the other when the condition is bilateral. The microscopic appearance in gynecomastia of testicular choriocarcinoma is seen in Figure 53, in that of extragenital choriocarcinoma in Figure 54, and the lesion resulting from treatment with stilbestrol in Figure 55. In these the epithelial proliferation is greater than in most unilateral lesions, but not in all, and the loose periductal tissue with inflammation and fibrosis of the stroma is essentially the same. Since the microscopic changes of unilateral gynecomastia cannot be distinguished from those of the bilateral type known to be associated with evident endocrine disorders, probably due to the influence of estrogens, it may be that estrogens also play a part in the pathogenesis of unilateral gynecomastia.

Theories on Unilateral Gynecomastia. All theories on unilateral gynecomastia are hypothetical and none is founded on hormonal assays. Although expressing doubt as to validity, Weber [4] cited Lacaille as having described 2 cases of unilateral acromegaly. Weber thought of unilateral gynecomastia as the result of "potential hemihypertrophy . . . . ready to be converted into actual unilateral hypertrophy or gigantism by hormonal stimulation." Later he and Atkinson [102] phrased his views somewhat differently, stating that in certain persons a portion of one side of the body forms a "local soil" differing from that of the other side, so that the two sides vary in their reaction toward a general stimulus, *e.g.*, endocrine disorder. Recently Weber [103] repeated his view to the effect that "just as one half of the body is occasionally bigger than its fellow, it is probable that one half may be *potentially bigger*, in the sense that certain organs in that half may respond more readily to hormonic or other stimulation than organs in the other half."

Richardson [50] emphasized the action of estrogenic hormones. Levi [104] objected vigorously to Richardson's interpretation, questioning the unilateral activity of hormones. Medvei [105] raised doubts as to the views of both writers and introduced the factor of constitution. He quoted Bauer as stating that there are two forms of gynecomastia, a constitutional form "due directly to a chromosomal imbalance" and a hormonal form in which "hereditary factors, when present, operate through the medium of the endocrine system." Enough has been said above to discredit these unsupported hypotheses.

Exophthalmos of hyperthyroidism may be unilateral and Pochin [106] has called attention to the fact that retraction of the upper lid, Dalrymple's sign, may also be unilateral. Fuller Albright, in a personal statement, explained unilateral gynecomastia as due to a dif-

ference in receptors for endocrine stimuli. Pochin, also in a personal communication, elaborated the views of Weber [103] and of Albright to the effect that the difference in receptors is not obvious when the endocrine stimulus is massive, but that slight stimuli may meet receptors somewhat more sensitive on one side than the other.

Pseudogynecomastia is only rarely unilateral. It is not clear that there is hormonal basis other than that occasionally operative in obesity.[7] Endocrine substances might be increased to such a slight degree that assays would not be delicate enough to reveal the change from normal, although by operating over long periods on sensitive receptors the stimulus might be effective.

Treatment

If treatment is required, it should be surgical. Deaver and McFarland [11] gave the indications for surgical treatment as anxiety, mortification, or pain. To these must be added environmental conditions which subject the breast to friction or injury and also the suspicion that the lesion is neoplastic.

Endocrine products are ineffectual in the treatment of gynecomastia. Young [107] treated 6 patients with "pituitary lactogenic substance," testosterone, and progesterone, without observable effect. Sullivan and Munslow [22] used methyl testosterone in 1 case unsuccessfully. Dunn [13] cited McCullagh and McGurl as having been successful in the local injections of male sex hormone but pointed out that there has been no confirmation over the course of 4 years and said that his own results were negative. Klinefelter, Reifenstein, and Albright [63] found that no favorable effects resulted from administration of estradiol dipropionate, progesterone, pregneninolone, or testosterone propionate.

Generally speaking, there is no reason for biopsy. The removal of the breast is a simple operation and a cross section of the lesion usually suffices to distinguish between gynecomastia and neoplasm, although microscopic examination gives the final decision. Depending on circumstances, the operation may be designed to remove mammary gland, areola, nipple, and skin, leaving enough skin to permit immediate closure, or it may be a subcutaneous resection of the mammary gland, leaving areola and nipple, as described by Webster.[16]

Summary

Definition

Gynecomastia is an enlargement of the mammary gland or glands of males due to proliferation of connective tissue, dense in the general stroma and often loosely arranged in periductal regions, together with

variable degrees of multiplication, elongation, or branching of ducts, or of all three, without formation of true acini, accompanied by periductal or more widespread infiltration of lymphocytes, plasma cells, large mononuclear cells, and occasionally eosinophils or neutrophilic polymorphonuclear cells, or both; secretion is frequently present in ducts, may be discharged spontaneously or manually expressed, but rarely if ever is it true colostrum or milk.

This definition excludes pseudogynecomastia, due to the deposition of fat in the mammary region, as well as suppurative or other essentially inflammatory processes, granulomatous lesions, and neoplasms, either benign or malignant.

Etiology

Gynecomastia is much more frequently unilateral than bilateral, and the unilateral lesions are about equally divided between the two sides. It may occur at almost any time of life, but is especially frequent in the third decade. Only rarely does it succeed directly upon the pubescent subareolar node. After reaching a certain size, which varies widely from case to case, growth ceases, but reduction in size is rare. Resolution, to the point of complete cicatrization, occurs in breasts with active proliferation, but is limited to small foci and does not indicate resolution of the whole lesion. There is no clear indication that true neoplasia supervenes.

When gynecomastia accompanies manifest endocrine disorders it is usually bilateral, but not always equal, and occasionally only one breast is enlarged. In such cases, epithelial proliferation is conspicuous, but it is no greater than that frequently seen in unilateral gynecomastia without demonstrated endocrine disturbance.

From present knowledge of assays of hormones, no generalizations can be made. In obvious endocrine disorders, hormonal imbalance is observed, but increase in estrogenic substances is frequent. Experimental results and observations on man, when correlated, point toward estrin as the important factor. Even though androgens may excite proliferation in the mammary gland of experimental animals, there is little if any evidence that they, of themselves, have the same effect in man. Unilateral enlargement may possibly be due to hormonal imbalance or hyperestrinism acting over a long time on receptors especially sensitive on the one side, but of such small proportions as not to be demonstrable by current methods of assay.

Trauma or irritation may draw attention to the lesion and may perhaps augment growth, but there is no satisfactory evidence that either one is causative, nor is there evidence that hereditary or constitutional

factors (other than those implied in certain endocrine disorders) play any significant part.

When treatment is required, it should be surgical. Results have not justified the use of endocrine products in treatment of gynecomastia.

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variable degrees of multiplication, elongation, or branching of ducts, or of all three, without formation of true acini, accompanied by periductal or more widespread infiltration of lymphocytes, plasma cells, large mononuclear cells, and occasionally eosinophils or neutrophilic polymorphonuclear cells, or both; secretion is frequently present in ducts, may be discharged spontaneously or manually expressed, but rarely if ever is it true colostrum or milk.

This definition excludes pseudogynecomastia, due to the deposition of fat in the mammary region, as well as suppurative or other essentially inflammatory processes, granulomatous lesions, and neoplasms, either benign or malignant.

Etiology

Gynecomastia is much more frequently unilateral than bilateral, and the unilateral lesions are about equally divided between the two sides. It may occur at almost any time of life, but is especially frequent in the third decade. Only rarely does it succeed directly upon the pubescent subareolar node. After reaching a certain size, which varies widely from case to case, growth ceases, but reduction in size is rare. Resolution, to the point of complete cicatrization, occurs in breasts with active proliferation, but is limited to small foci and does not indicate resolution of the whole lesion. There is no clear indication that true neoplasia supervenes.

When gynecomastia accompanies manifest endocrine disorders it is usually bilateral, but not always equal, and occasionally only one breast is enlarged. In such cases, epithelial proliferation is conspicuous, but it is no greater than that frequently seen in unilateral gynecomastia without demonstrated endocrine disturbance.

From present knowledge of assays of hormones, no generalizations can be made. In obvious endocrine disorders, hormonal imbalance is observed, but increase in estrogenic substances is frequent. Experimental results and observations on man, when correlated, point toward estrin as the important factor. Even though androgens may excite proliferation in the mammary gland of experimental animals, there is little if any evidence that they, of themselves, have the same effect in man. Unilateral enlargement may possibly be due to hormonal imbalance or hyperestrinism acting over a long time on receptors especially sensitive on the one side, but of such small proportions as not to be demonstrable by current methods of assay.

Trauma or irritation may draw attention to the lesion and may perhaps augment growth, but there is no satisfactory evidence that either one is causative, nor is there evidence that hereditary or constitutional

factors (other than those implied in certain endocrine disorders) play any significant part.

When treatment is required, it should be surgical. Results have not justified the use of endocrine products in treatment of gynecomastia.

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DESCRIPTION OF PLATES

PLATE 54

FIG. 1. Bilateral gynecomastia in a young man with entirely male characteristics.

FIG. 2. Unilateral gynecomastia in a young Negro of marked masculinity.

FIG. 3. Elongation, distention, and branching of ducts. × 10. Acc. no. 92355.

Karsner

Gynecomastia

PLATE 55

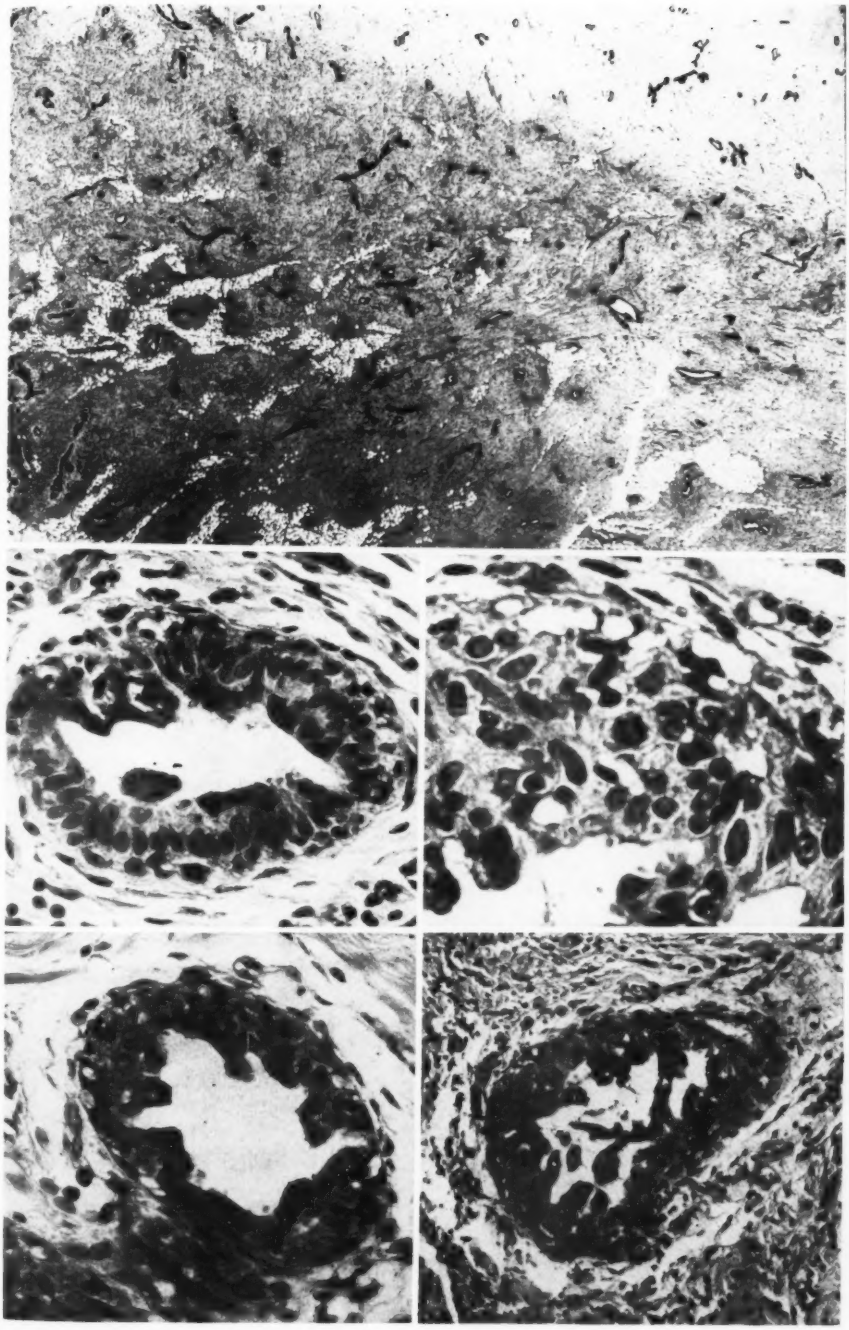
FIG. 4. Marked increase in number of ducts. × 10. Army Institute of Pathology Acc. no. 85377.

FIG. 5. Mitotic figure in basal layer of the epithelium of a duct. × 354. Acc. no. 67499.

FIG. 6. Mitotic figure in middle layer of the epithelium of a duct. × 624. Acc. no. 98307.

FIG. 7. Mitotic figure in luminal layer of the epithelium of a duct. × 354. Acc. no. 94770.

FIG. 8. Multiplication of epithelium of duct with formation of epithelial sprouts projecting into the lumen. There are fibroblasts and mononuclear cells in the periductal tissue. × 142. Acc. no. 71373.



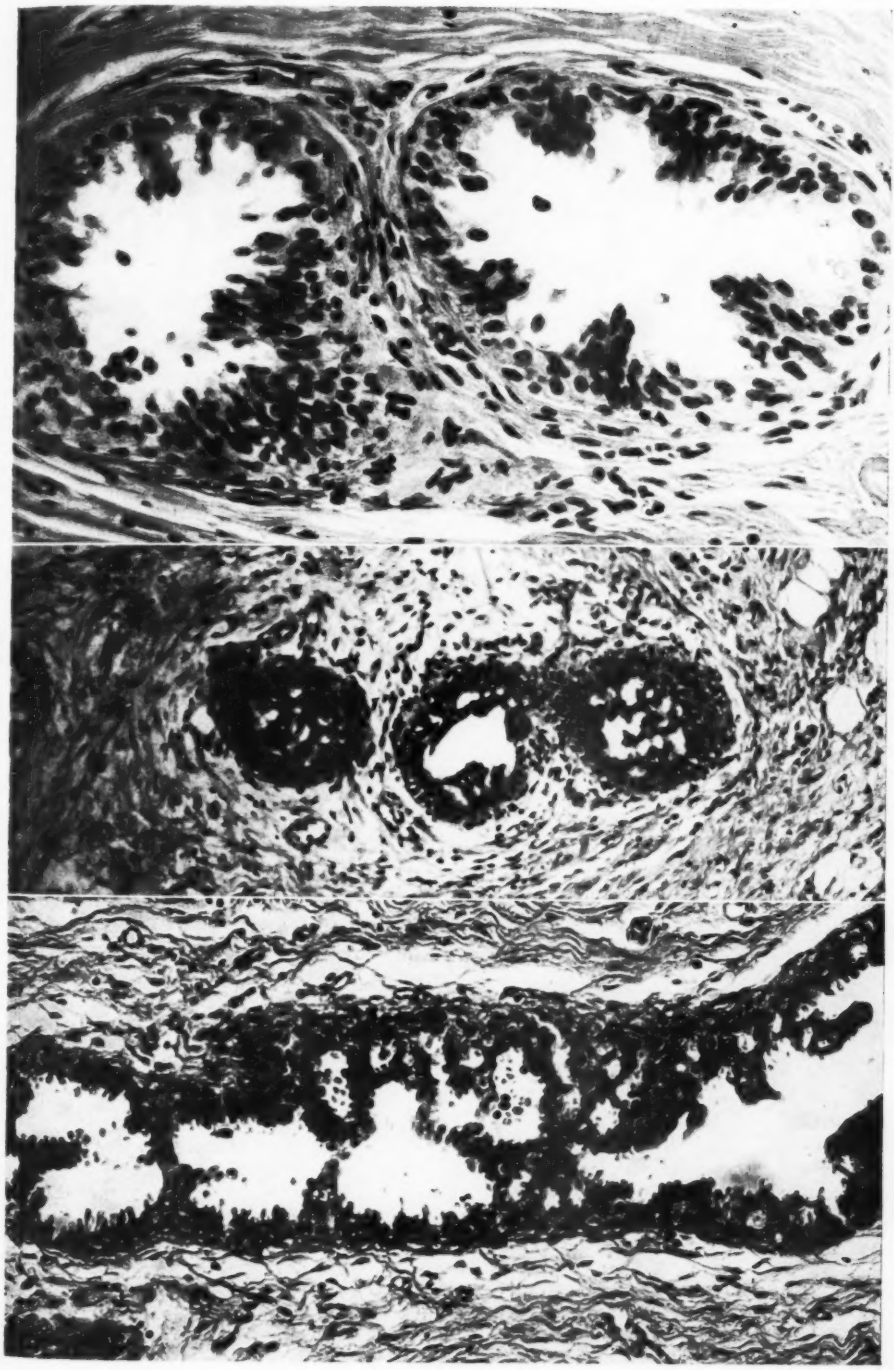
Karsner Gynecomastia

PLATE 56

FIG. 9. Elongation of epithelial cells toward cylindrical form, with intraductal sprouts. × 354. Acc. no. 67449.

FIG. 10. Multiplication of epithelium of duct and fusion of sprouts to form locules, viewed in cross section. × 142. Acc. no. 78890.

FIG. 11. Multiplication of epithelium of duct and fusion of sprouts to form locules, viewed in longitudinal section. × 215. Acc. no. 99887.



Karsner Gynecomastia

Plate 57

Fig. 12. Multiplication of epithelium of duct, filling the lumen. × 215. Acc. no. 78890.

Fig. 13. Elongation and branching of a duct with budding at the ends of two branches. × 102. Acc. no. 106546.

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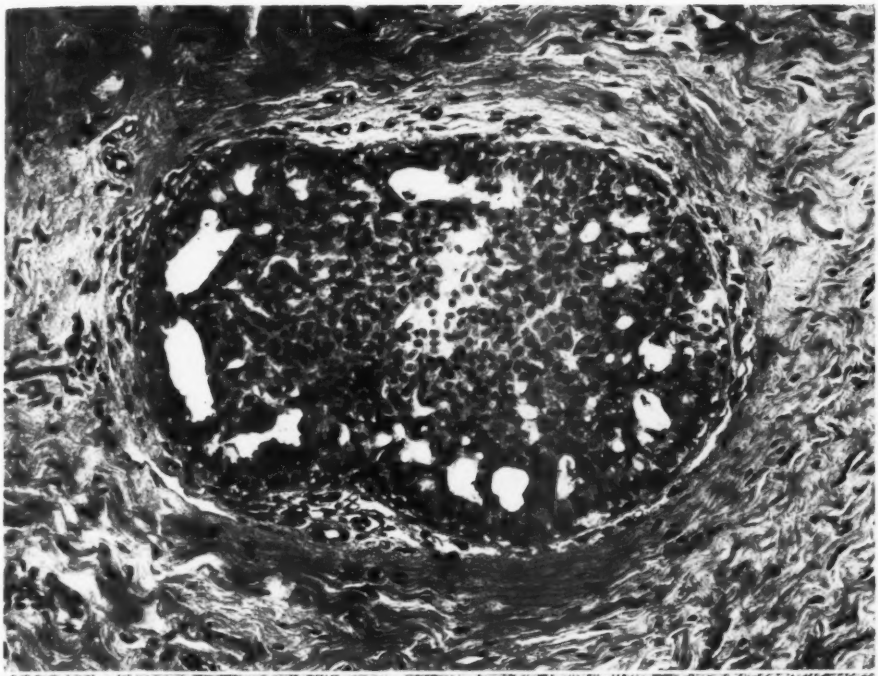

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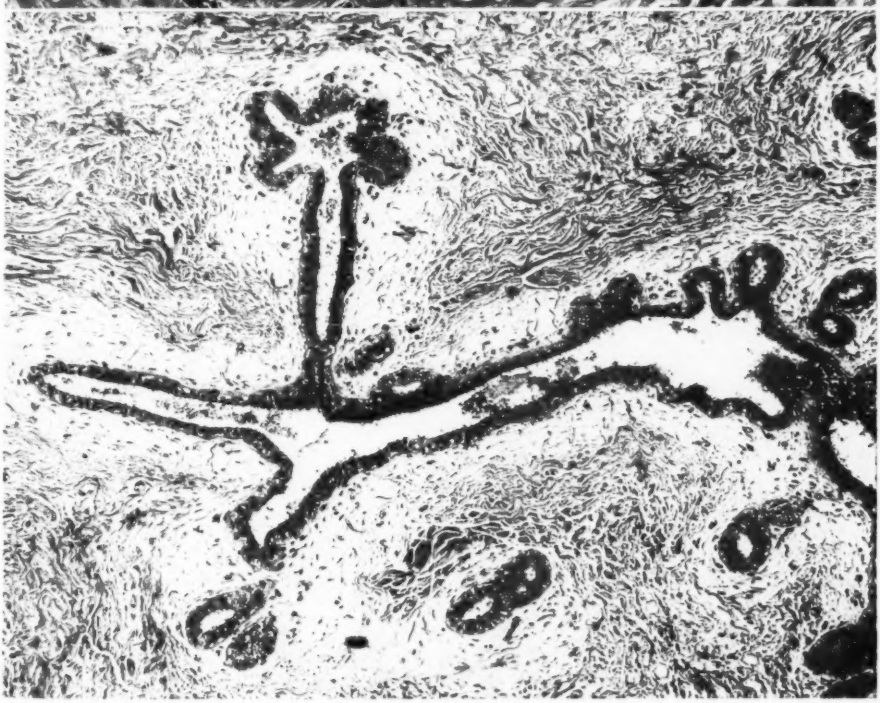

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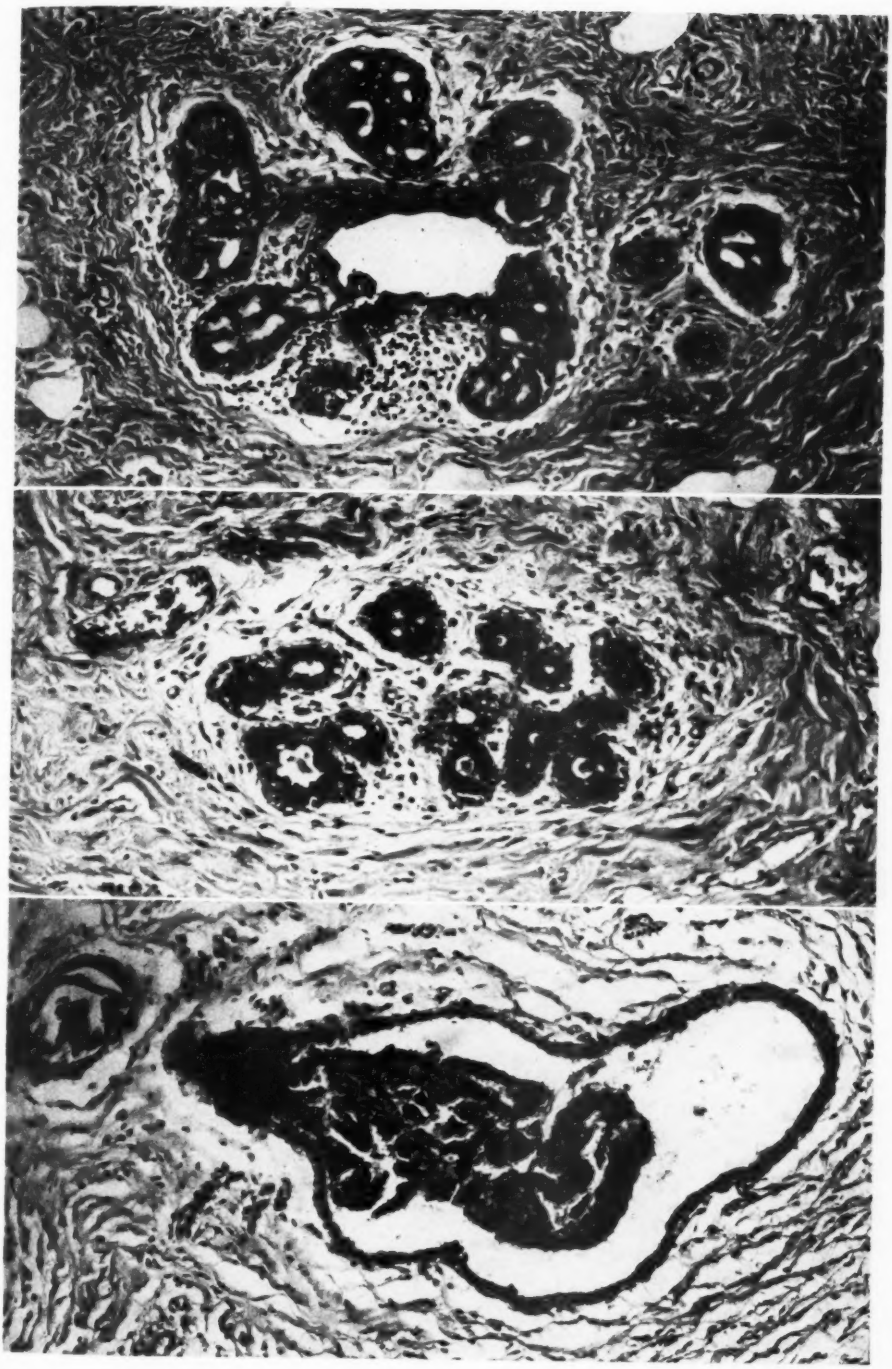
PLATE 58

FIG. 14. Branching of duct with budding, as seen in cross section. There is a notable mononuclear cellular infiltration. × 142. Acc. no. 78302.

FIG. 15. Cross section of buds of a duct at a level not including the duct of origin, suggesting lobules but with multiple lining layers. × 142. Acc. no. 94960.

FIG. 16. Papillae with connective tissue cores in a slightly distended duct. × 150. Acc. no. 86111.



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Gynecomastia

PLATE 59

FIG. 17. Papilla with connective tissue core in a markedly distended duct which contains acidophilic precipitate and desquamated epithelial cells. × 142. Acc. no. 99858.

FIG. 18. Branching papillae with connective tissue cores in a distended duct. × 109. Acc. no. 94551.

FIG. 19. Deformity of outline of duct with connective tissue growth projecting into lumen. × 142. Acc. no. 72255.

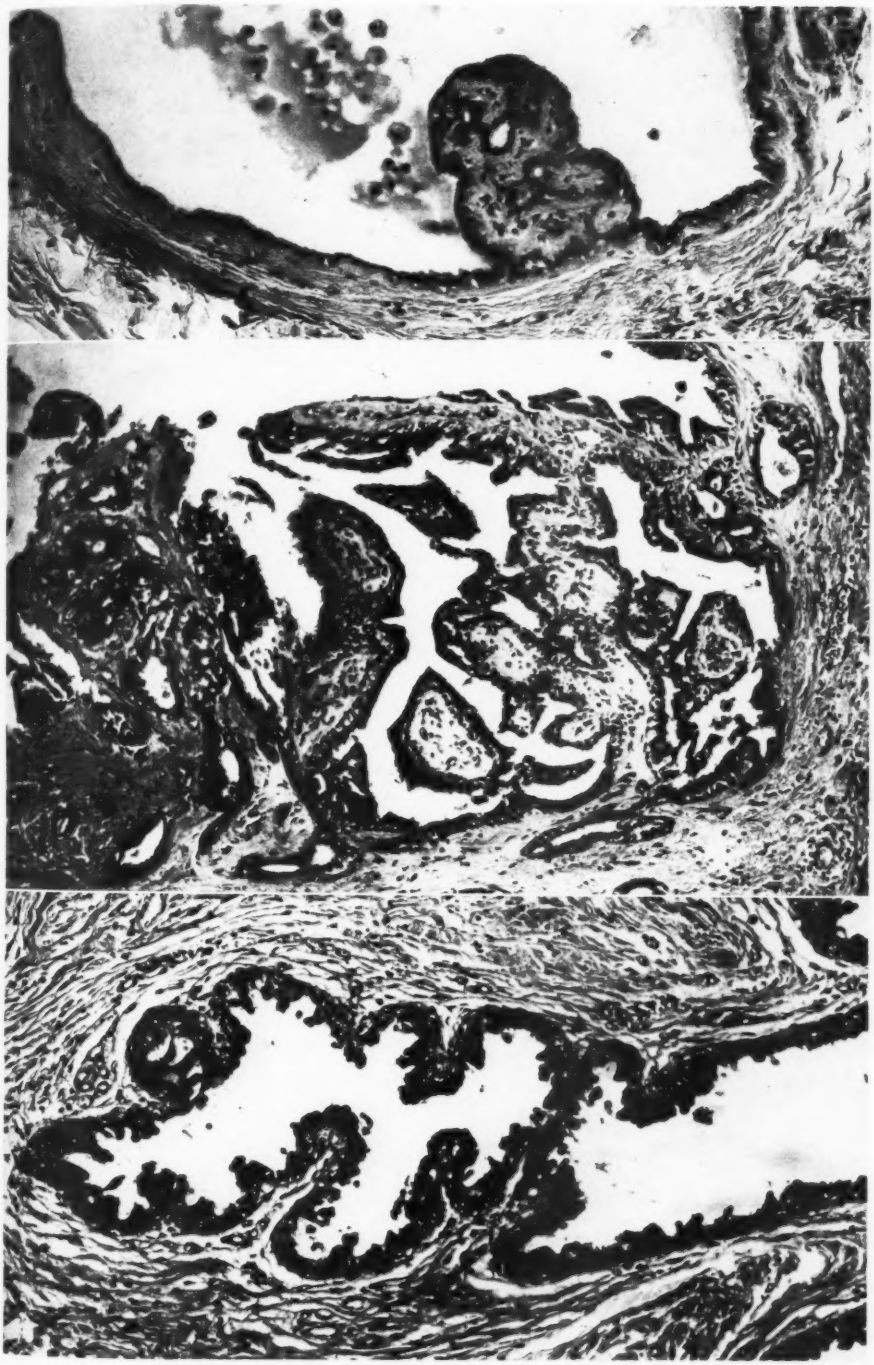

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Plate 60

Fig. 20. Deformity of outline of duct with connective tissue growth projecting into lumen. Granular acidophilic precipitate in lumen and periductal infiltration of mononuclear cells. × 142. Acc. no. 70626.

Fig. 21. Duct of nipple with apparent deformity due to line of section. Periductal infiltration with mononuclear cells. × 124. Acc. no. 82300.

Fig. 22. Deformity of duct with connective tissue growth forming large, blunt projections into lumen. No whorling of connective tissue in the projecting masses. × 109. Acc. no. 93620.

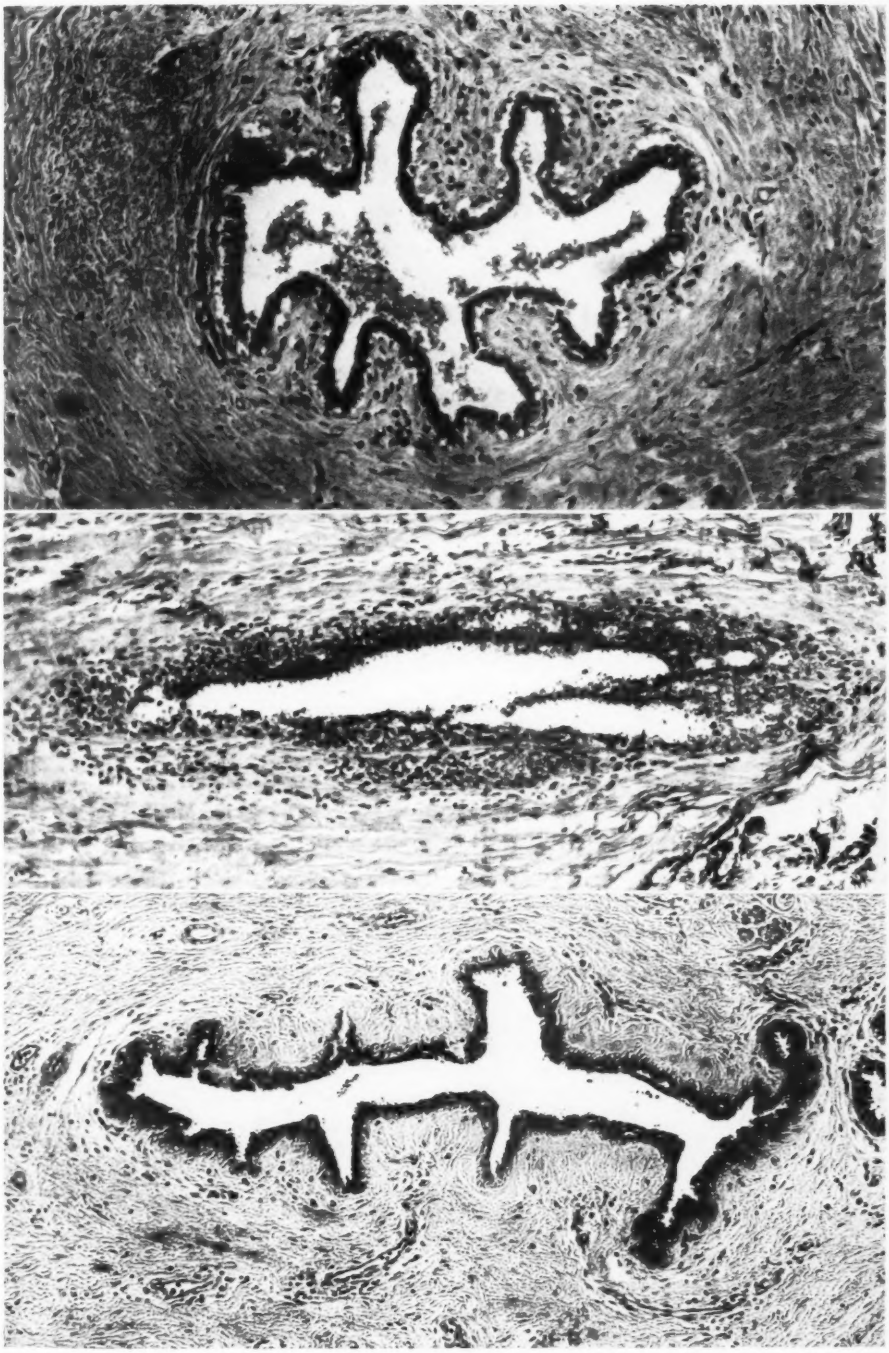

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Plate 61

Fig. 23. Acidophilic, finely granular precipitate in lumina of ducts, the epithelium of which has multiplied. × 354. Acc. no. 76253.

Fig. 24. Duct of nipple containing finely granular, basophilic precipitate and desquamated cells. Suggests colostrum but is devoid of fat. × 110. Acc. no. 84727.

Fig. 25. Mucinous cylindrical epithelial cells. × 280. Acc. no. 93817.

Fig. 26. Squamous cell metaplasia in a slightly distended duct. × 142. Acc. no. 60405.

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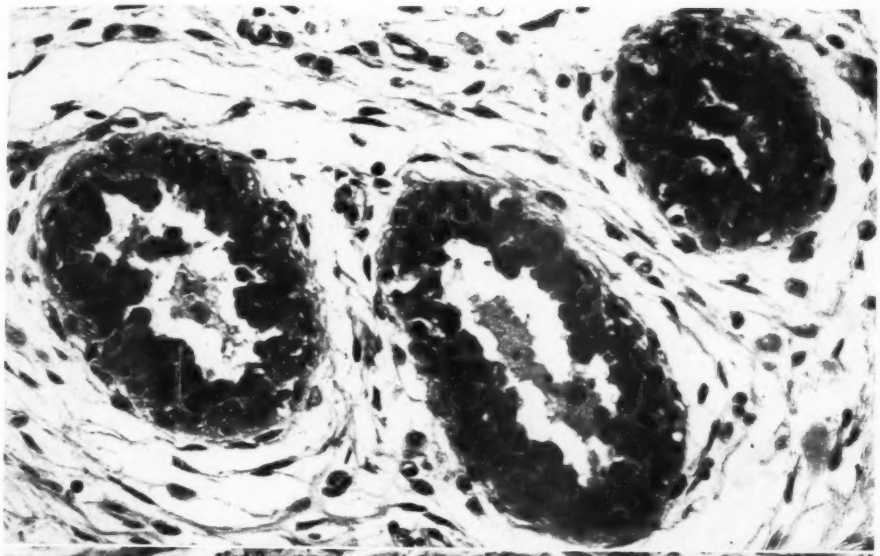

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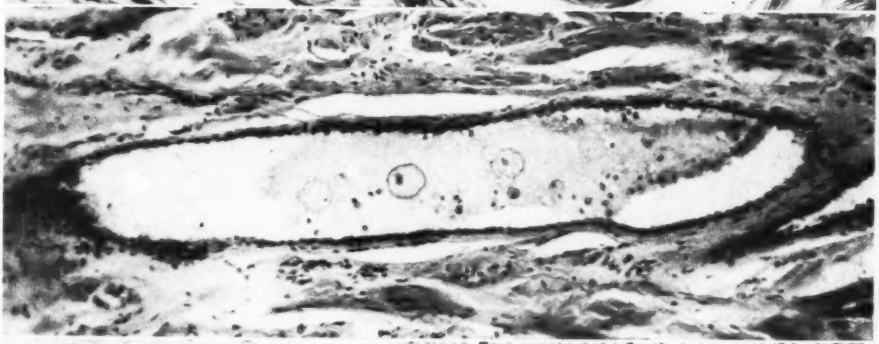

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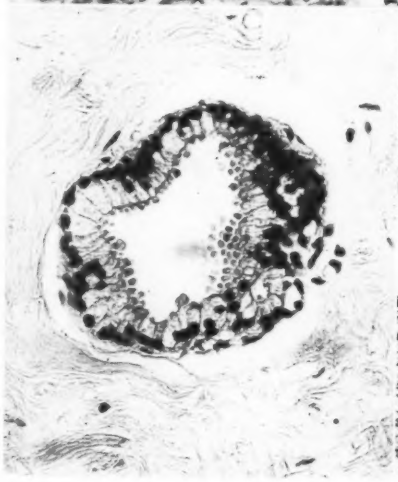

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Gynecomastia

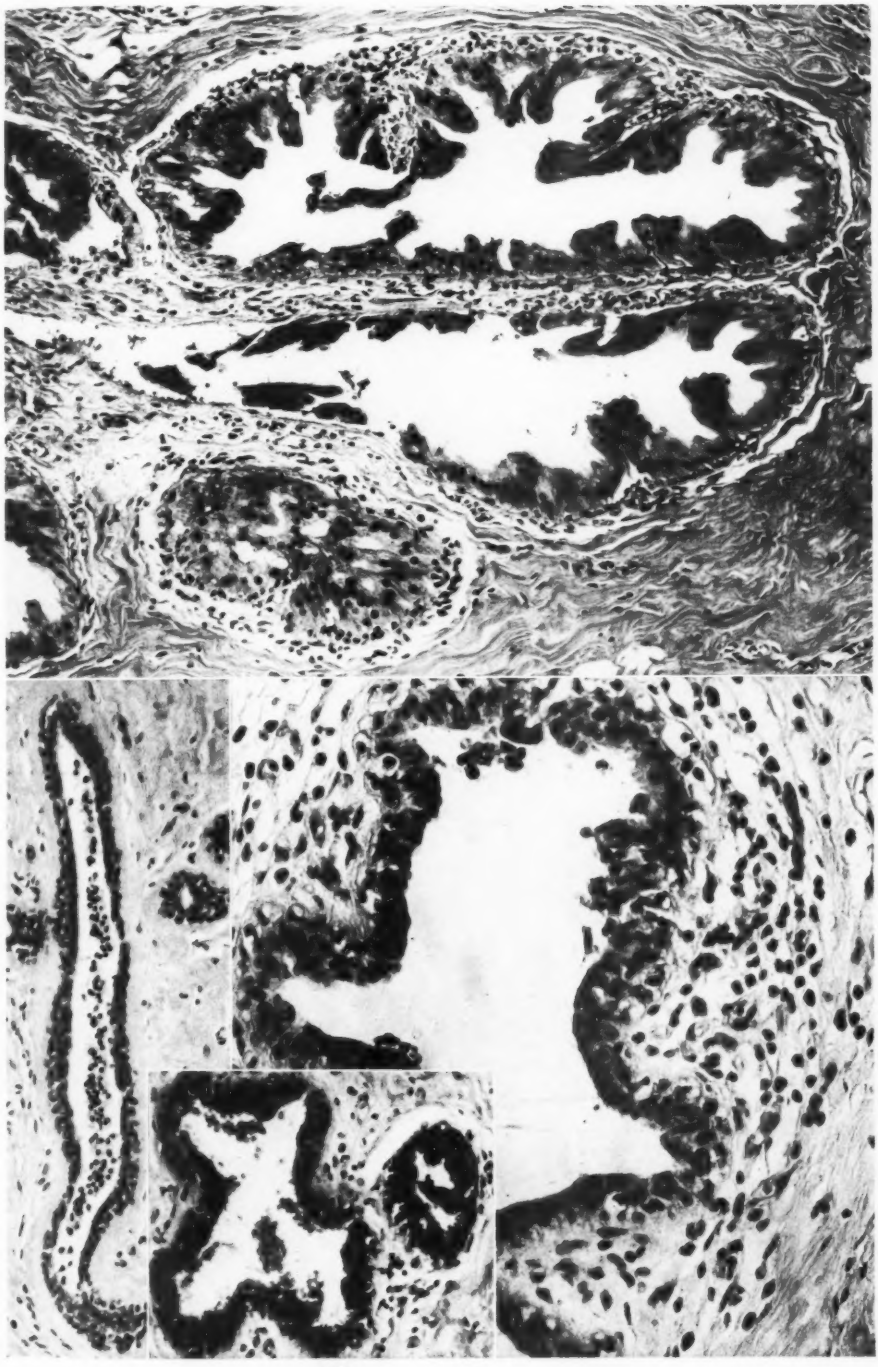
PLATE 62

FIG. 27. Ducts lined by multiple layers of large epithelial cells with acidophilic cytoplasm. × 142. Acc. no. 67499.

FIG. 28. Duct with conglutinated erythrocytes in lumen. × 179. Acc. no. 60022.

FIG. 29. Duct with lymphocytes in lumen. × 179. Acc. no. 75838.

FIG. 30. Infiltration of lymphocytes, plasma cells, and large mononuclear cells in loose periductal tissue. × 354. Acc. no. 98568.



Karsner Gynecomastia

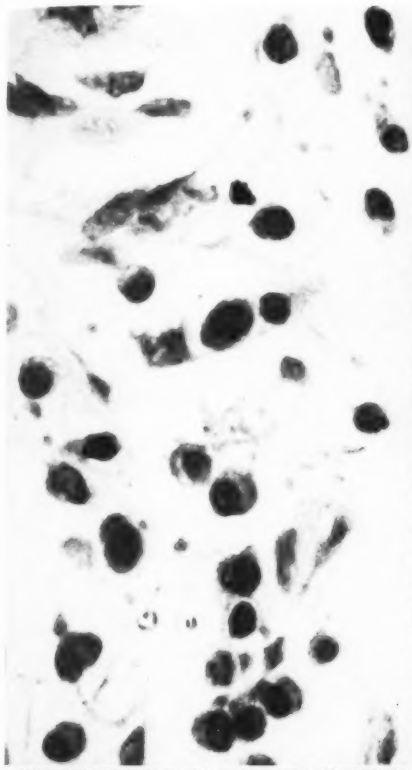
Plate 63

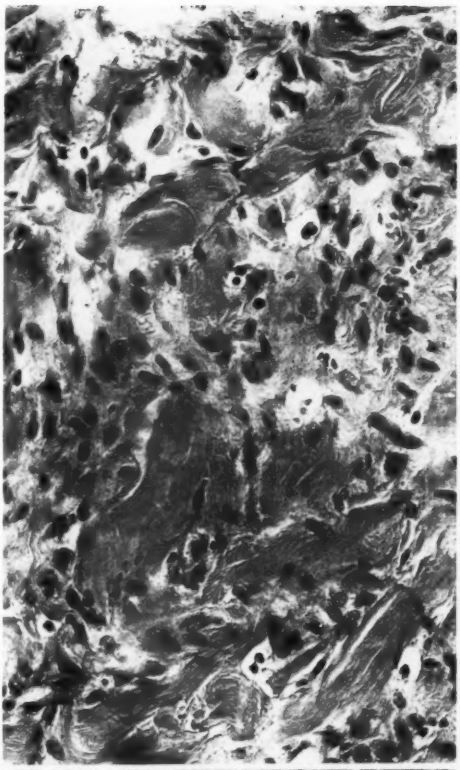
Fig. 31. Infiltration of lymphocytes, plasma cells, and large mononuclear cells in loose periductal tissue. × 900. Acc. no. 78549.

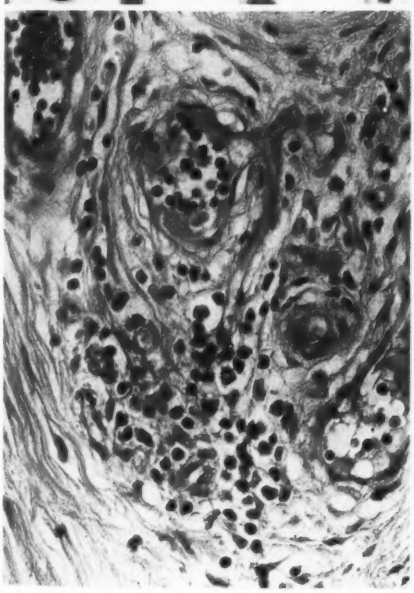
Fig. 32. Perivascular infiltration of mononuclear cells in stroma of mammary gland. × 354. Acc. no. 97872.

Fig. 33. Numerous fibroblasts in dense stroma of mammary gland. × 252. Acc. no. 65257.

Fig. 34. Proliferating capillaries in moderately loose periductal tissue. × 142. Acc. no. 76999.







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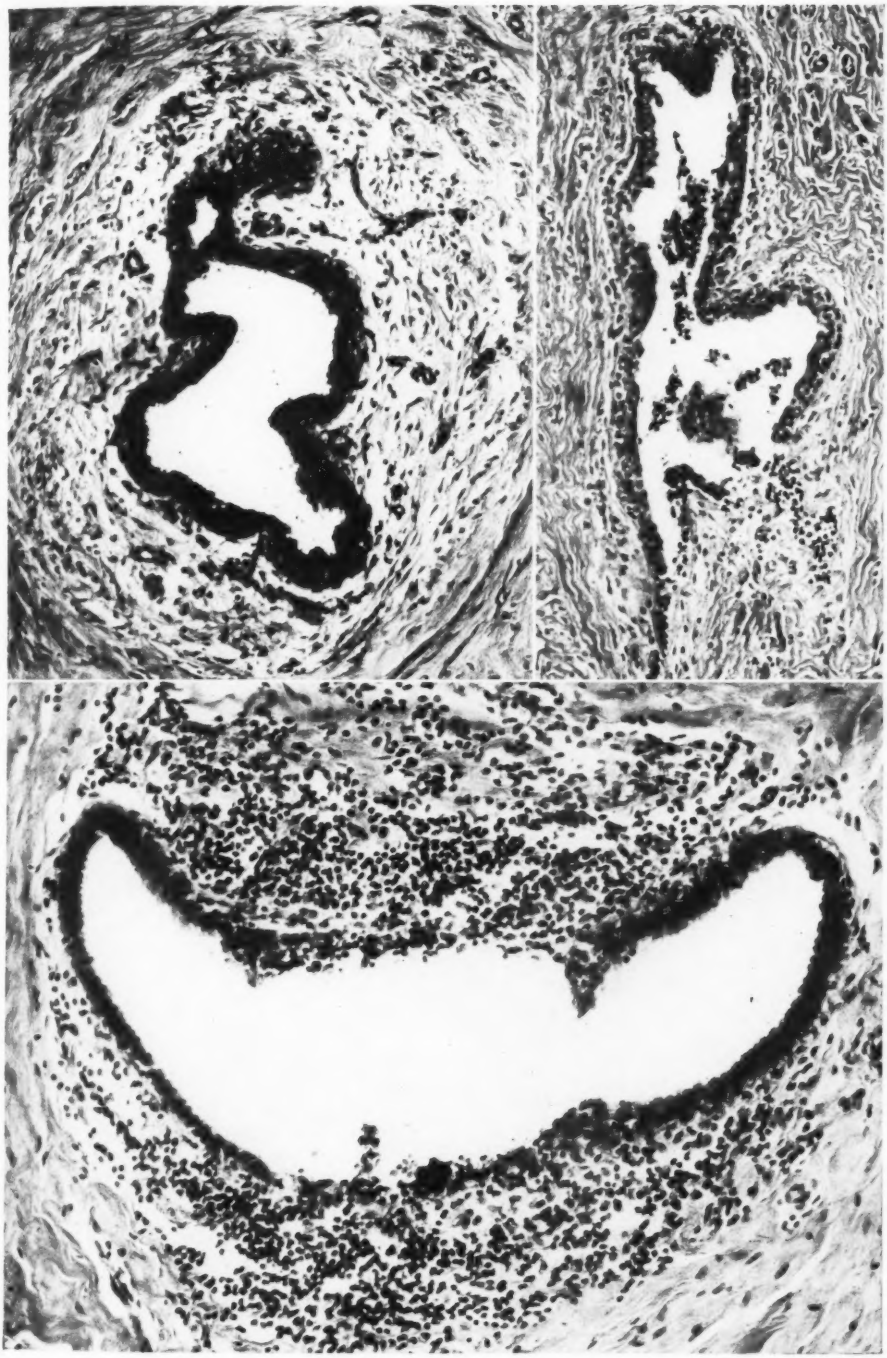
Gynecomastia

Plate 64

Fig. 35. Capillaries, fibroblasts, and mononuclear cells in loose periductal connective tissue. × 142. Acc. no. 96406.

Fig. 36. Exudate around and within a duct, with desquamation of lining epithelium. × 142. Acc. no. 84978.

Fig. 37. Massive exudation around a duct with slight exudation within the duct. Partial denudation of epithelial lining. × 215. Acc. no. 86302.



Karsner Gynecomastia

PLATE 65

FIG. 38. Localized proliferation of ducts, not encapsulated. × 10. Acc. no. 83595.

FIG. 39. Detail of Figure 38, to show ductal proliferation, epithelial multiplication, and infiltration of mononuclear cells into loose periductal tissue. × 142. Acc. no. 83595.

FIG. 40. Elongation of intraductal epithelium, suggestive of intraductal carcinoma, but without pleomorphism or invasion. × 179. Acc. no. 73774.

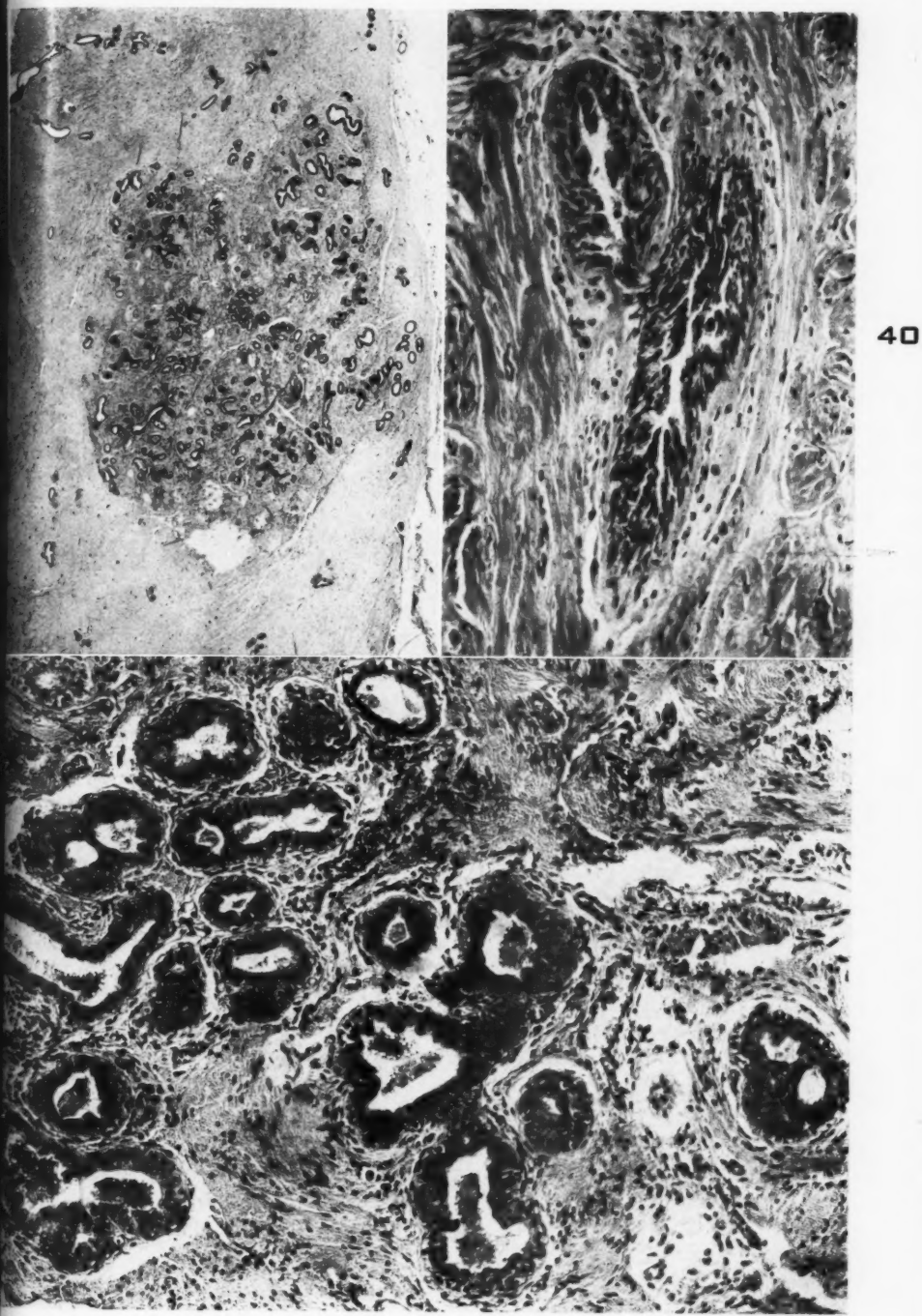


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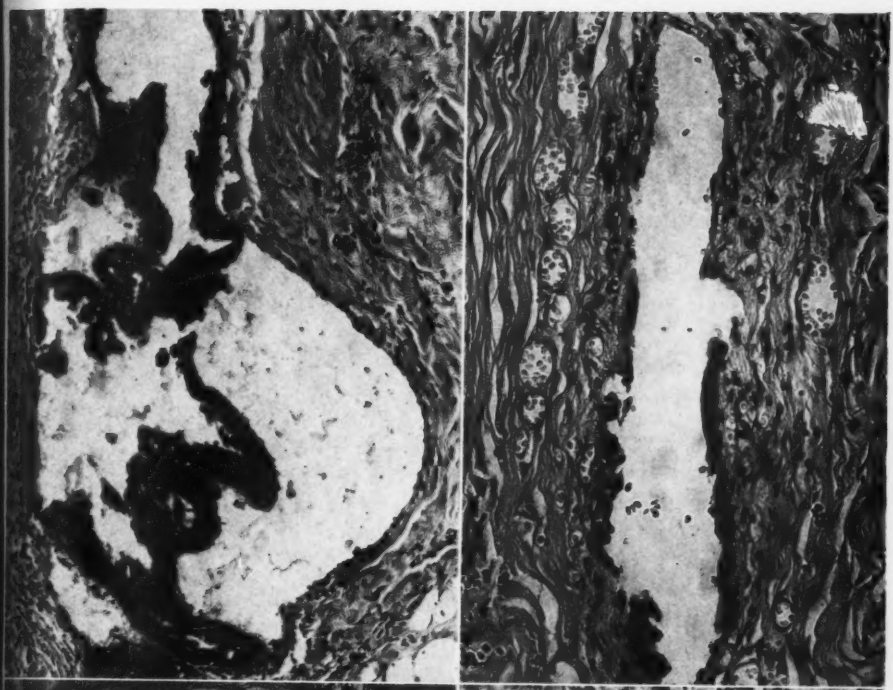
PLATE 66

FIG. 41. Desquamation of lining epithelium of duct, with partial denudation of wall and basophilic, finely granular precipitate. × 117. Acc. no. 86095.

FIG. 42. Partial denudation of lining epithelium. Many distended capillaries in periductal connective tissue. × 215. Acc. no. 92335.

FIG. 43. Partial denudation of lining epithelium of duct with surrounding inflammation and condensed mass of epithelium at one side. × 142. Acc. no. 86095.

FIG. 44. Condensation of ductal epithelium within a duct surrounded by granulation tissue. × 179. Acc. no. 67605.



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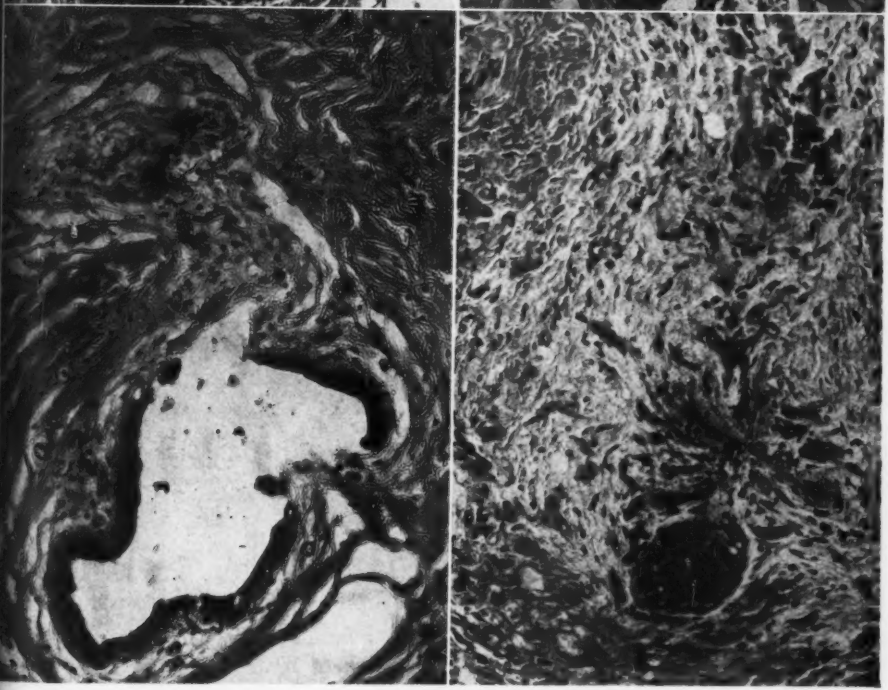

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Plate 67

Fig. 45. Almost complete disappearance of ductal epithelium. Mass of granulation tissue surrounding the remains of the lumen. × 252. Acc. no. 99820.

Fig. 46. Disappearance of lining epithelium and preservation of hyaline basement membrane of a duct, with a surrounding mass of granulation tissue. × 179. Acc. no. 67499.

Fig. 47. Complete disappearance of ductal lumen, with a central mass of granulation tissue surrounded by fibroblastic tissue. × 173. Acc. no. 88324.

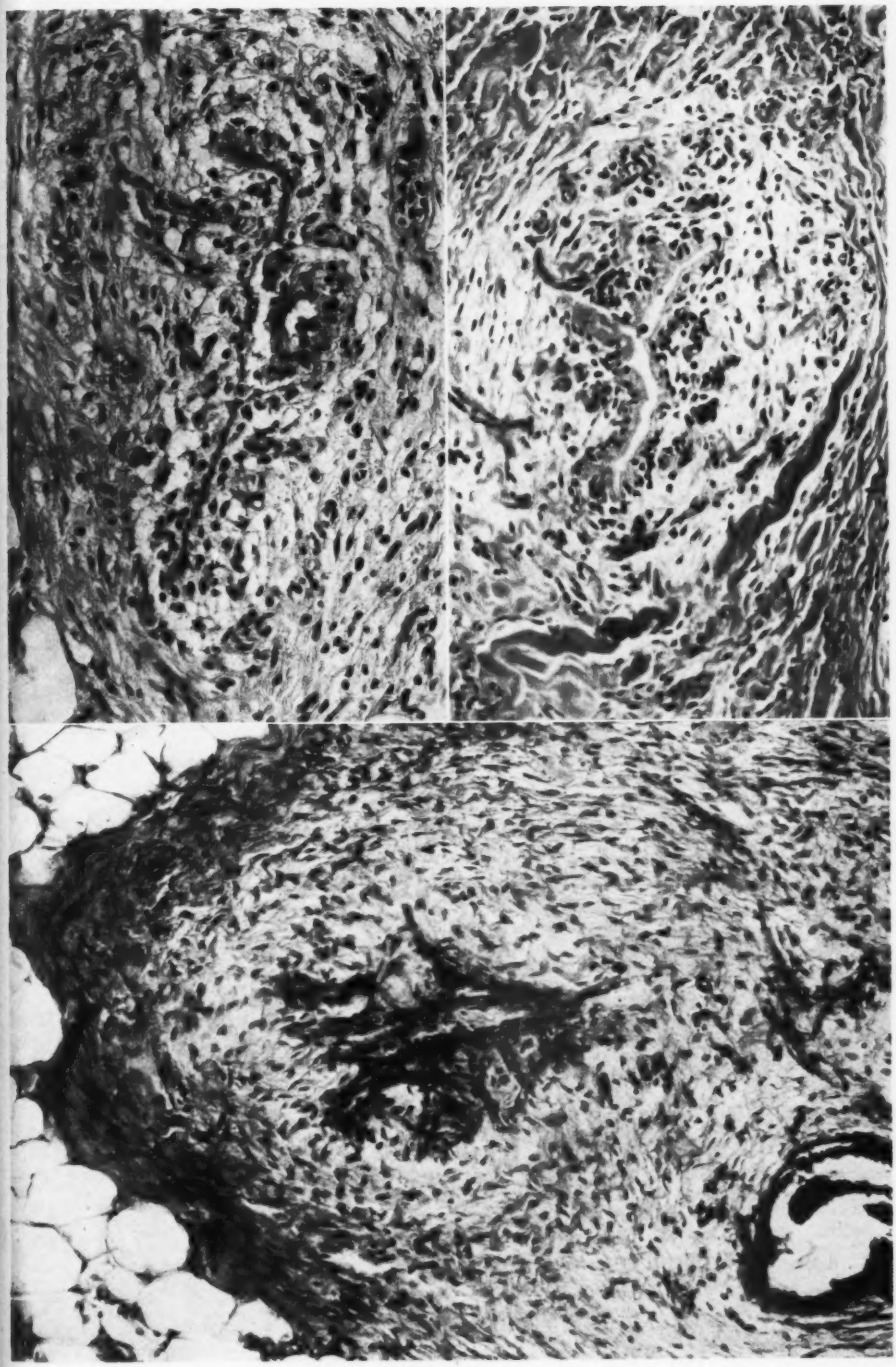

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PLATE 68

FIG. 48. Tightly coiled sudoriferous sweat gland, not abnormal. × 109. Acc. no. 77430.

FIG. 49. Loosely coiled, relatively inactive, apocrine gland. × 109. Acc. no. 81678.

FIG. 50. Apocrine gland in late stage of activity, with granular secretion in lumina. × 109. Acc. no. 75250.

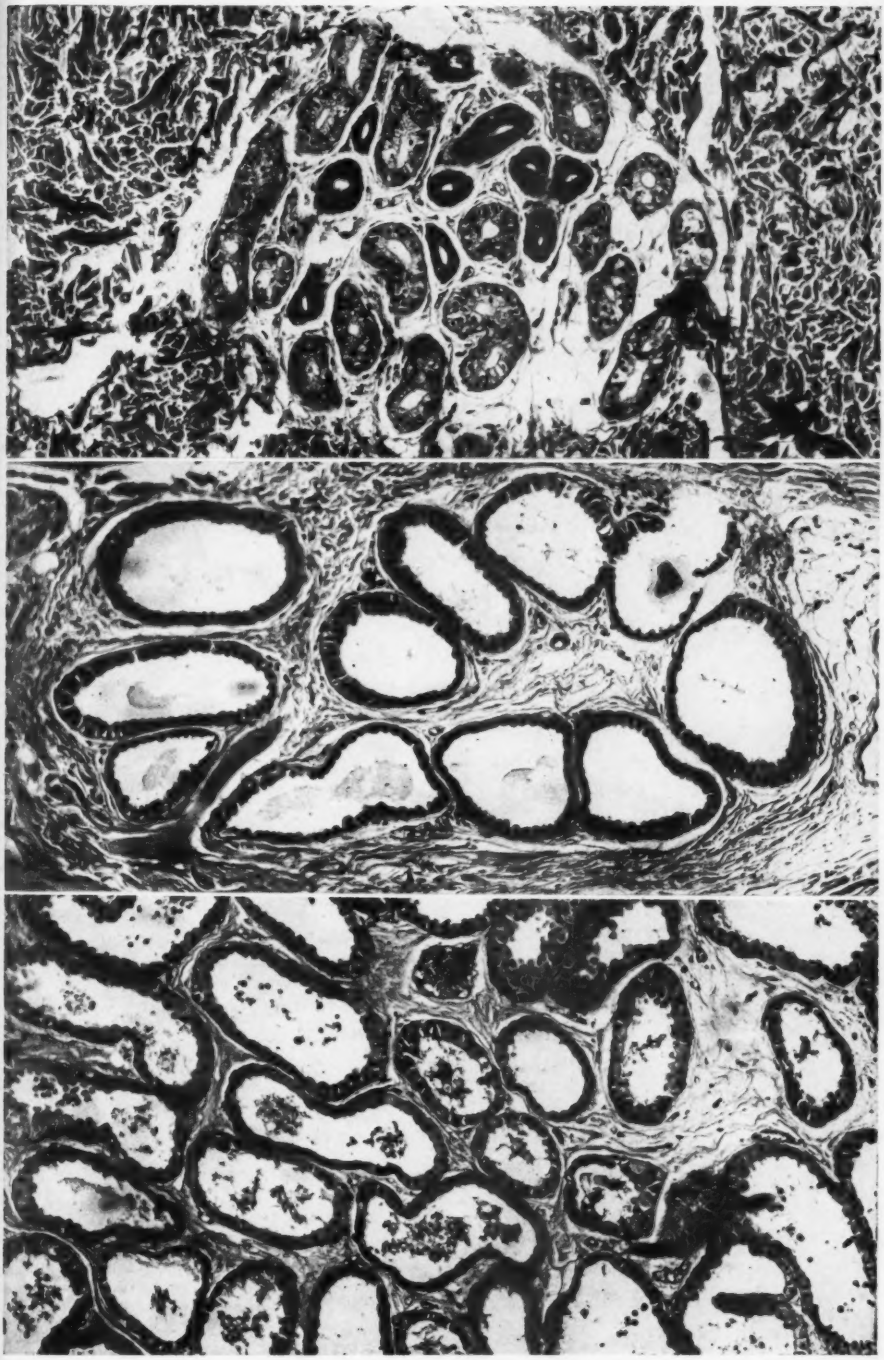

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PLATE 69

FIG. 51. Subacute dermatitis with dyskeratosis and also infiltration of mononuclear cells in papillary and upper reticular layers. × 142. Acc. no. 89770.

FIG. 52. Subacute dermatitis with crust formation and infiltration of mononuclear cells in papillary and upper reticular layers. × 142. Acc. no. 89770.

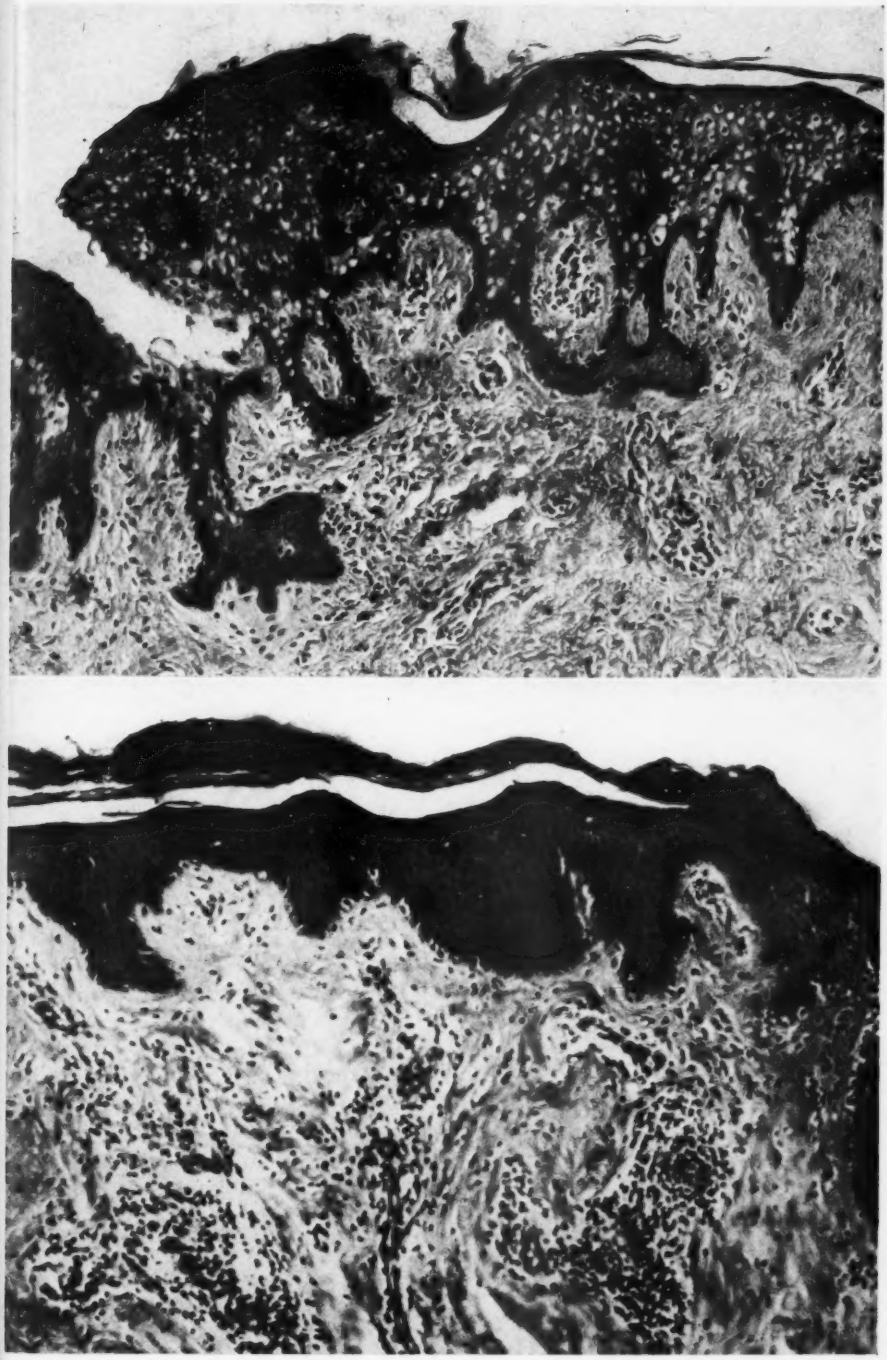

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Plate 70

Fig. 53. Multiplication of lining epithelium, and infiltration of mononuclear cells in periductal tissues. Case of choriocarcinoma of testis. × 142. Acc. no. 93056.

Fig. 54. Multiplication of lining epithelium and infiltration of mononuclear cells in loose periductal tissue. Case of choriocarcinoma originating in thorax (Laipply and Shipley [60]). × 142. Western Reserve University, Inst. Path., A 8014.

Fig. 55. Multiplication of lining epithelium and infiltration of mononuclear cells in loose periductal tissue. Prolonged administration of diethylstilbestrol in a case of carcinoma of the prostate, a white man, 70 years old. × 160. Acc. no. 75980.

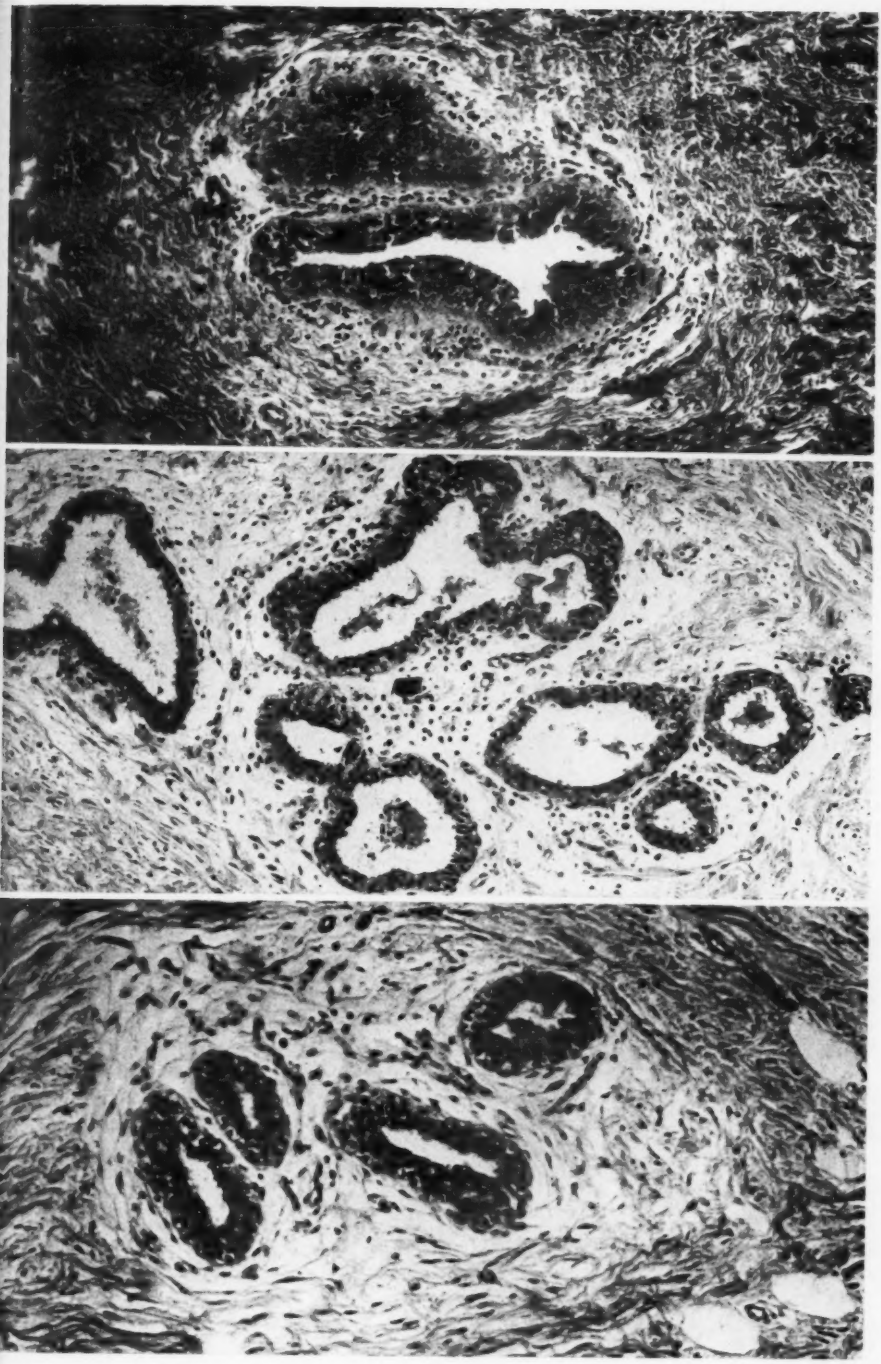

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Plate 71

Fig. 56. Photomicrograph by Ansco color process to show mucoid degeneration in loosely arranged, inflamed, periductal connective tissue. × 135. Acc. no. 97594.

Fig. 57. Photomicrograph by Ansco color process to show apocrine gland in secretory phase with acidophilic globules in cytoplasm. Of note is the myoepithelium. × 350. Acc. no. 77829.

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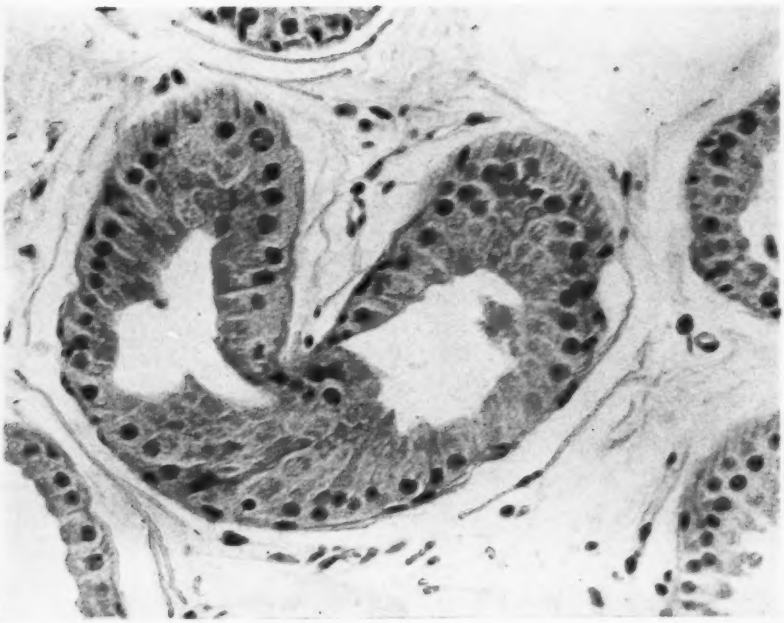

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STUDIES ON THE EARLY CHANGES IN THE LIVERS OF RATS TREATED WITH VARIOUS TOXIC AGENTS, WITH ESPECIAL REFERENCE TO THE VASCULAR LESIONS

II. The Histology of the Rat's Liver in Allyl Formate Poisoning *

A. Rosin, M.D.,† and L. Doljanski, M.D.

(From the Department of Experimental Pathology, The Hebrew University, Jerusalem, Palestine)

The present report is one of a series dealing with the initial effect of various toxic agents on the vascular system and the parenchyma of the liver.[1] It is concerned with the early changes occurring in allyl formate (2-propenyl methanoate, $HCOO\text{-}CH_2\cdot CHCH_2$) intoxication.

Material and Methods

Observations were made on 34 albino rats whose average weight was about 100 gm. Allyl formate was administered by the intraperitoneal route. Thirty rats received a single injection of 0.015 cc. per 100 gm. of body weight of allyl formate diluted in 2 cc. of Ringer's solution; they died or were sacrificed 1 to 2, 3 to 6, and 24 hours after administration of the drug. A group of 4 rats which survived 24 hours were given a second dose of 0.015 cc. of allyl formate per 100 gm. of body weight; all of these latter animals died 2 to 7 hours after receiving the second dose.

Pieces of liver were fixed in a 4 per cent solution of formaldehyde, in Carnoy's fluid, and in Zenker's solution, and were blocked in celloidin-paraffin. Sections, 6 μ in thickness, were stained with hematoxylin and eosin, Heidenhain's iron-hematoxylin, and with Mallory's anilin blue collagen stain. Frozen sections stained with sudan III were used for the study of fats.

Results

A. Findings in Rats Examined 1 to 2 Hours after Administration of Allyl Formate

Macroscopically, the livers appeared enlarged and dark red‡ 1 to 2 hours after administration of allyl formate. On microscopic examination the most striking features were the strong capillary congestion and the damage of the wall of the sinusoidal capillaries (Figs. 1–3). The

*Received for publication, March 20, 1945.

† Working under a grant in aid given by Mrs. J. H. Stodel, Cape Town, South Africa.

‡ The gross changes of the livers were the same in all series of experiments with a single dose of allyl formate. For this reason no further mention will be made of the gross appearance of the organ.

congestion of the sinusoids was most marked in the peripheral third of the lobule, though occasionally it extended throughout the whole lobule. The sinusoids were greatly widened and engorged with red blood cells; the liver cell cords often appeared compressed to small thread-like bands. The capillary wall was usually swollen, hyaline, and often disrupted. The Kupffer cells were frequently detached; their cytoplasm was sometimes strongly eosinophilic and the nuclei pyknotic. In many places the distended sinusoidal clefts were entirely devoid of endothelial lining and the fragments of the capillary wall were found among the red blood cells between the liver cell cords. In other places, where no rupture of the capillary wall occurred, the walls of the sinusoidal capillaries were in close contact with the liver cells, the pericapillary spaces being only rarely or not at all visible. There were no signs of pericapillary edema.

The vascular damages were not limited to the sinusoidal capillaries but also affected the portal and sublobular veins. Their endothelium was often swollen or desquamated and their wall hyaline and structureless. The connective tissue around the portal canals was edematous and the accompanying lymphatics were greatly distended (Fig. 4). Occasionally the portal and sublobular veins were surrounded by areas containing extravasated erythrocytes (Fig. 5) and eosinophilic granular material, resembling precipitated albumin. In the walls of the portal veins the fibers were often loosened and there were erythrocytes between them.

At this stage the epithelial parenchyma of the liver showed no appreciable alterations, though a certain number of liver cells situated in the zones of intensive capillary damage displayed the signs of early hydropic degeneration. They were somewhat swollen and their cytoplasm contained either several well defined smaller vacuoles or one or two larger ones (Fig. 6). These vacuoles usually appeared to be empty. In some cases, however, they contained a homogeneous transparent material which stained faintly pink with eosin and occasionally one or several erythrocytes could be observed within these vacuoles. There was no fat in the liver cells. The nuclei of the liver cells were normal in size and shape, with well defined nuclear membrane.

B. Findings in Rats Examined 3 to 6 Hours after Administration of Allyl Formate

Three to six hours after administration of allyl formate the damages in the portal and sublobular veins were more accentuated. Many of them were now surrounded by rather broad hemorrhagic areas. The sinusoidal capillaries, especially those in the peripheral parts of the

lobule, were destroyed, and fragments of the capillary lining were often found in the distended sinusoidal clefts together with pinkish staining granular material, detached Kupffer cells, and erythrocytes. The rupture of the capillaries resulted in the formation of small blood pools.

The hydropic vacuolization of the liver cells may reach an extensive degree at this stage, and in many cases it affected the whole of the epithelial parenchyma. The presence of fat could not be demonstrated in the vacuoles; many of them contained homogeneous material which stained uniformly pink with eosin, and also red blood corpuscles, single or in small groups.

In rat 17 the pathologic picture was especially illustrative. In this animal the ingestion of erythrocytes by the liver cells had attained an extreme degree. The trabecular tracery of the liver lobules was greatly obscured in the peripheral areas, which often presented a completely uniform, sieve-like appearance (Fig. 7). Examination at higher magnification showed that most of the liver cells in these areas were swollen and possessed a large vacuole. The vacuoles were sometimes empty, but frequently contained red blood cells as well as a hyaline material, which stained slightly pink with eosin (Figs. 8–13). The numbers of ingested erythrocytes varied from a few to a great many. When they were numerous, they filled the vacuoles completely and were massed together to form larger conglomerates. The individual corpuscles within the conglomerates were well defined and well stained. When the number of ingested erythrocytes was small, the liver cells retained their characteristic shape and size. As the inclusions increased in volume the nuclei were pushed aside and the protoplasmic rim became narrower. Finally the cytoplasm was reduced to a very thin ring staining reddish brown with hematoxylin and eosin, and the flattened nuclei appeared as sickle-shaped bodies. The distended hepatic cells were pressed so closely one to another that the intercellular spaces between them appeared either very small or completely obliterated.

C. Findings in Rats Examined 24 Hours after Administration of Allyl Formate

The portal and sublobular veins of rats examined 24 hours after administration of allyl formate often exhibited severe changes, amounting occasionally to complete destruction of the vessel walls. But also at this stage the vessels were not uniformly damaged, and side by side with markedly affected veins numerous others were encountered appearing to be intact. Perivascular edema was not present, although the perivascular hemorrhagic areas were often extensive. The changes in capillaries were similar to those described in the foregoing paragraph.

At this stage the liver cells in the peripheral areas of the lobule showed the first definite signs of necrosis. They were swollen and their homogeneous cytoplasm stained reddish brown with eosin. Many of the nuclei became poorly defined or invisible, others were irregularly shrunken and fragmented. The outlines of the cells could often be distinguished only with difficulty. The necrotic areas were sharply limited. The hepatic cells in the central parts of the lobule were always well preserved.

D. Findings in Rats Which Received Two Injections of Allyl Formate

Rats surviving 24 hours were injected with a second dose of 0.01 cc. of allyl formate. They died 2 to 7 hours after administration of the second dose.

The livers were swollen, congested, brownish red, and showed an interlacing network of red lines.

The most striking feature of the liver changes in rats of this series was the extensive hemorrhages associated with widespread parenchymal necrosis. The portal and sublobular veins were constantly surrounded by broad hemorrhagic zones of irregular outline which often extended as wide tracts from one portal area to the next (Fig. 14). The walls of the veins situated in the hemorrhagic areas were greatly thickened and had a hyaline appearance. They were often devoid of endothelial lining and contained erythrocytes between the collageneous fibers. Sometimes the veins were completely destroyed and their former position could only be recognized by the accompanying arteries and bile ducts.

The perivascular hemorrhages pushed aside the liver tissue surrounding the affected vessels, thus completely destroying the normal architecture of the liver. There were often no remnants of hepatic tissue left in the hemorrhagic areas. In other cases only the parenchymal cells disappeared in the affected zones, whereas the mesenchymal supporting stroma of the organ remained. Red blood corpuscles, detached Kupffer cells, and isolated liver cells were mingled with the reticular framework.

In places where the hemorrhages did not attain this extreme degree and did not replace the parenchyma around the periportal tracts, massive and widespread necrosis of the peripheral liver cells could be observed. It developed around the portal vessels, extended to the midzonal regions, and fused with similar necrotic areas of other lobules (Fig. 15). The cytoplasm of the liver cells appeared granular and

stained brownish red with eosin, probably because of intense imbibition with hemoglobin. The liver cells became swollen and their boundaries disappeared progressively. At first the nuclei persisted as structureless bluish brown masses; later they became dimly visible or were absent. Ingested red blood cells or their remnants could often be distinguished in the necrotic liver cells. Sometimes the liver cells showed a more advanced degree of necrosis with breaking up of cell bodies and complete obliteration of the intercellular spaces. Most of the central areas of the lobules showed no obvious departure from normal and persisted as islands among the necrotic tissue or hemorrhage; in addition there was often a comparatively narrow, unaffected zone, sometimes of a breadth of only one or two liver cells, bordering the portal venules. The necrotic areas were sharply defined, there being no gradual transition from these to areas of healthy tissue; immediately adjacent to cells showing complete necrosis were cells hardly affected at all. The nuclei of remaining liver cells were often very large; binucleated cells were numerous; no mitotic figures were seen. There was a slight leukocytic infiltration.

Discussion

Piazza [2] was the first to report on the effect of allyl formate on the liver. Working with mice, rabbits, cats, and dogs, he studied the changes produced in the livers after doses of 0.05 gm. of allyl formate per kg. of body weight administered by the subcutaneous route. He gave no detailed description of the histologic changes but mentioned the vacuolization and necrosis of the peripheral liver cells, as well as widespread hemorrhages in the necrotic areas.

Eppinger, Kaunitz, and Popper [3] and Popper [4] studied the effect of allyl formate, after subcutaneous and oral administration, on the livers of dogs. They noted considerable sinusoidal congestion and the detachment of the walls of sinusoidal capillaries from the liver cell cords; the distended pericapillary spaces were found to be filled with an eosinophilic granular material and sometimes contained erythrocytes. At a more advanced stage there was widespread necrosis of parenchymal cells, the changes leading ultimately to cirrhosis of the liver. Similar alterations were observed by Popper [5] in the livers of salamanders after injection of allyl formate under the skin of the back.

Eppinger and co-workers [3] considered allyl formate to be a specific capillary poison and attributed the liver necrosis occurring in intoxication with allyl formate to capillary injuries, which interfere with the normal exchange between the parenchymal cells and the blood. The fact that allyl formate increases the permeability of the blood vessels

was demonstrated by Roller and Schober.[6] They injected the salamander intravenously, first with uranin solution and then with allyl formate. After the allyl formate injection the uranin solution leaked through the vessel walls and could be demonstrated photographically as fluorescent bands accompanying the blood vessels. Raff and Abrahamczik [7] reported that the permeability of animal membranes (ox gut) is increased under the influence of allyl formate and allylamine.

The view advocated by Eppinger and his associates [3] that allyl formate acts primarily as a vascular poison was opposed by Heinemann,[8] working in Aschoff's laboratory. She injected rats and rabbits with single or repeated doses of allyl formate. In rats, she observed the congestion of sinusoids, but no damage of the capillaries, no pericapillary edema, and no necrosis of the liver cells. In rabbits she noted periportal necrosis with hemorrhages in the necrotic areas, but no major capillary damages, and no detachment of the capillary walls from the liver cell cords. Heinemann claimed that in allyl formate intoxication the liver cell injuries precede the vascular damage, and concluded that allyl formate is not a capillary but a liver cell poison.

The problem of the nature of the liver changes in allyl formate poisoning is of especial interest for the following reason: In 1934, Rössle [9] described as "serous hepatitis" an inflammatory process in the liver, which affects the capillaries, increases their permeability, and leads to leakage of plasma into the intercellular spaces. The original injury to the capillaries is followed by dissociation and occasional disappearance of the liver cells as well as by development of collagenous fibers within the extravasated plasma. Rössle regarded this condition as the first and obligatory stage of those morbid processes in the liver which finally lead to cirrhosis of the organ. "Serous inflammation" may also involve other organs, according to this author. This concept was later adopted by Eppinger and his associates.[3] They went much further, considering "serous inflammation" as the almost universal pathognomonic basis for the great variety of parenchymatous damages in most organs. As far as the liver is concerned, their assumption is based almost exclusively on the observations made in animals poisoned with allyl formate, since, according to them, the changes observed in allyl formate intoxication and in serous hepatitis are completely identical. They considered allyl formate intoxication as an experimentally produced "serous hepatitis." The concept of "serous inflammation" has been prominent in the German literature during the past decade, but has been accepted with some reserve in the United States (Keschner and Klemperer,[10] Karsner,[11] Lichtman,[12] Moon [13]).

In analyzing our results, it may be stated that allyl formate produces in the livers of rats a series of profound changes which involve both the parenchyma and the vascular system. The retrograde processes in the parenchyma of the organ are invariably preceded by vascular lesions.

Even at the earliest stages investigated (1 to 2 hours after injection) the damage of sinusoidal capillaries in the livers of rats treated with

allyl formate is very pronounced. These alterations are not limited to disturbance in the permeability of the capillary wall, as assumed by Eppinger and his associates,[3] but are of a more far-reaching character. The capillary damage produced by allyl formate consists in definite disorganization and often in complete destruction of the walls of the sinusoidal capillaries in the periphery of the lobule. Not the pericapillary edema, but the disappearance of the capillary lining and the hemorrhages into the parenchyma are the constant and characteristic features of the lesions here described. The vascular changes just described are not confined to the lining of the sinusoidal capillaries but are similarly marked in the branches of the portal and sublobular veins. The swelling and disorganization of the endothelium of the intima, hyalinization of the media, as well as mural and perivascular edema, can be regularly observed. These changes ultimately lead to the complete destruction and rupture of the vessel wall, causing extensive and widespread hemorrhages around the portal tracts. The surrounding parenchyma becomes flooded with extravasated blood.

The changes observed in the liver cells may be summarized as follows: The definite lesions of the liver parenchyma appear at a later stage than those of the vascular system. In experiments of 1 to 2 hours' duration, *i.e.*, at a stage when the destruction of the sinusoidal capillaries is already most pronounced, the liver cells show no evidence of injury, except for some slight signs of hydropic degeneration. The hydropic degeneration becomes very intense 3 to 6 hours after the administration of the drug. In addition to hemal fluid the liver cells at this stage are found to contain in their cytoplasm large numbers of red blood corpuscles. However, even in the areas of strongest hydropic degeneration there is no necrosis of liver cells at this time; the nuclei of the liver cells are well preserved throughout. Necrosis of parenchymal cells becomes apparent only 24 hours after injection of allyl formate. The cells affected are all situated in the peripheral parts of the lobules, whereas the central regions are always well preserved.

The results of the present investigation indicate quite clearly that allyl formate, a drug which possesses a pronounced capacity to provoke intense and widespread necrosis of the liver parenchyma and which, after repeated administrations, may produce cirrhosis of the liver,[3,4] is a powerful vascular poison. The sequence of hepatic changes in rats treated with allyl formate appears to indicate that allyl formate affects primarily the vascular system.

The fact that the capillary lesions precede the retrograde changes in the liver cells, the local coincidence of both vascular and paren-

chymal alterations, as well as the special character of the earlier parenchymal injuries (hydropic vacuolization, ingestion of erythrocytes), all point to a causal relationship between the vascular lesions and the liver cell changes. From the above account it would appear that the primary intense damage of the capillaries creates conditions which favor or provoke retrograde processes in the liver cells, and that these retrograde changes take place in an internal environment altered by the breakdown of the blood-tissue barrier.

It is to be noted that the liver changes caused by allyl formate bear much resemblance to the changes which we [1] found in rats treated with urethane, another substance with definite vessel-damaging properties. Both poisons attack the capillary walls and the walls of the large veins; both provoke the breakdown of the barrier between blood and parenchyma, an event resulting in hemorrhages into the parenchyma; the alterations of the liver cells, too, are of a similar nature in both types of poisoning.

The effects in allyl formate intoxication differ from those in urethane poisoning in that in the former the vascular damages clearly precede the alterations of the parenchymal cells, whereas in the latter the vascular and parenchymal damages coincide. Moreover, there is a distinct difference in the degree of the vascular lesions. They are much more pronounced in rats treated with allyl formate than in rats treated with urethane. In the case of urethane we are dealing in the first place with disturbances in the permeability of the capillary wall, while allyl formate produces changes amounting to complete breaking up of the capillary system. The moderate changes in the vascular system of the livers of animals treated with urethane are accompanied by little advanced liver cell changes; the far-reaching destruction of the blood vessels in allyl formate poisoning are attended with extensive necrotic processes in the liver parenchyma.

The comparison of events in allyl formate and urethane poisoning appears of interest, as it seems to indicate a quantitative relationship between the vascular and parenchymal lesions. When a milder vascular poison is applied, the parenchymal damages are slight; the administration of a strong vascular poison is followed by extensive parenchymal necrosis. This comparison would tend to show that the condition of the liver cells is essentially dependent on the condition of the vascular wall, and that the degree of the parenchymal damage is a function of the degree of vascular injury.

The observations made in these investigations may be added to those which strengthen the view that with some poisons alterations of the vascular wall are concerned in the genesis of necrosis of the liver cells.

This assumption does not, of course, exclude the possibility of a direct effect on the liver parenchyma of the substance administered. This possibility cannot be evaluated on the basis of the present study and requires further investigation.

Summary

Intraperitoneal administration of allyl formate causes, in the livers of white rats, severe and widespread damage of sinusoidal capillaries and portal veins, followed by extravasation of plasma and formed elements of the blood into the parenchyma. At a stage when injury of the sinusoidal capillaries is already very pronounced, there is still little evidence of change in the liver cells, except for signs of beginning hydropic degeneration. The hydropic degeneration of the hepatic cells progresses and some hours later reaches an extreme degree. The liver cells in the periphery of the lobule contain, at this stage, in addition to hemal fluid, erythrocytes in large numbers. Continued action of the poison causes extensive hemorrhage into the periportal areas, as well as necrobiosis of the liver cells in the peripheral and midlobular zones.

The results of the foregoing observations strengthen the view that the condition of the liver cells is essentially dependent upon the condition of the vascular wall. The data available are, however, not sufficient to exclude a direct action on the liver parenchyma of the substance administered.

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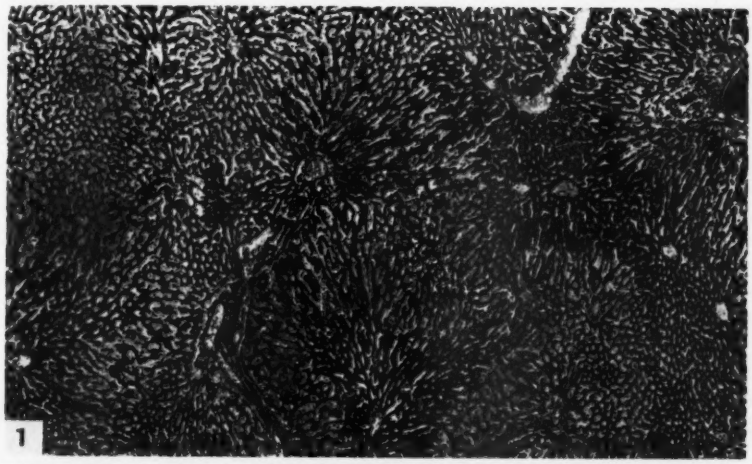
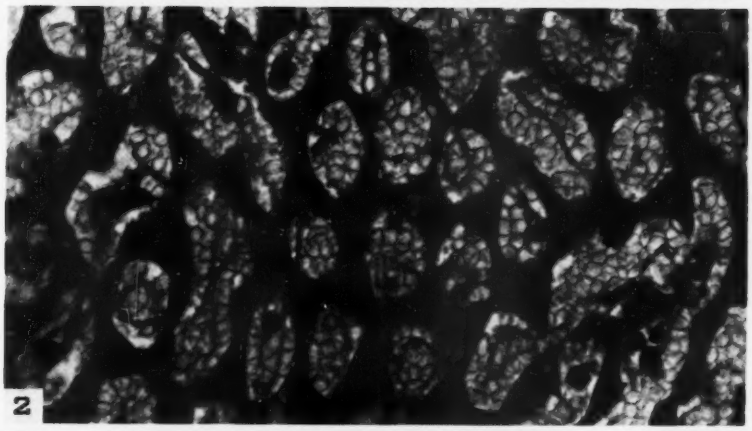
DESCRIPTION OF PLATES

(All figures were taken from hematoxylin and eosin preparations.)

PLATE 72

FIGS. 1–3. All from rat 83, which was killed 2 hours after the administration of allyl formate. Extensive sinusoidal congestion and rupture of capillaries. Figure 1, × 59; Figure 2, × 580; Figure 3, × 1400.





Eosin and Doljanski Rat's Liver in Allyl Formate Poisoning

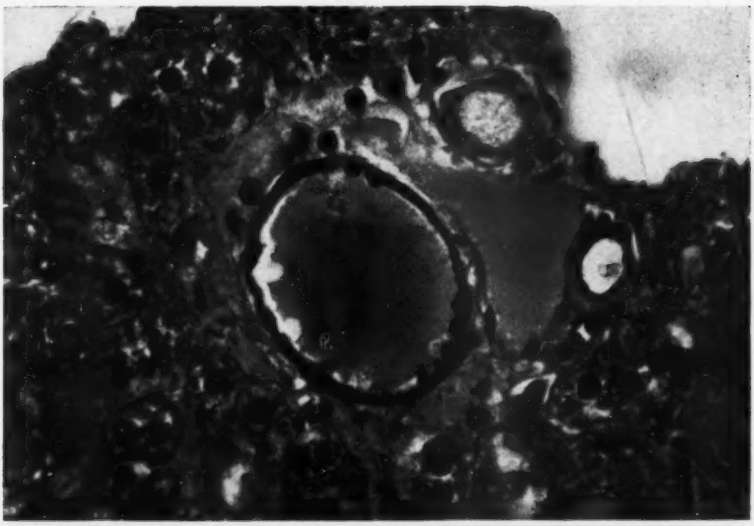
PLATE 73

FIG. 4. From rat 70, which died 1 hour after the administration of allyl formate. Marked edema around a portal vein. × 620.

FIG. 5. From rat 78, which died 1½ hours after the administration of allyl formate. Hemorrhage about a portal vein. × 500.

FIG. 6. From rat 76, which died 1 hour after the administration of allyl formate. Beginning hydropic degeneration of the hepatic cells; the vacuoles contain homogenous pinkish-staining material; in addition, in one liver cell (c) there is a single erythrocyte. × 1700.

4



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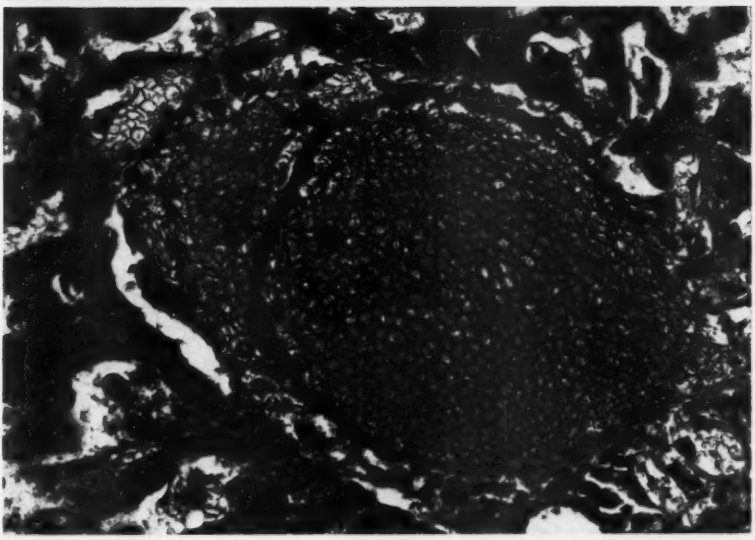

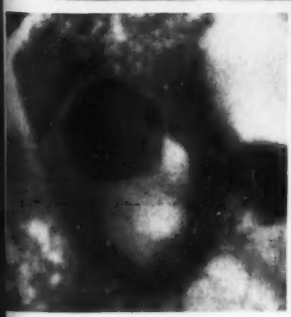

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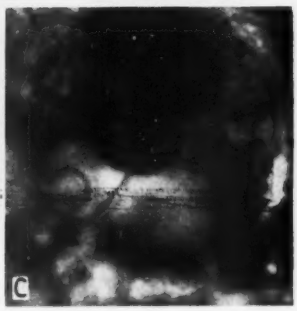


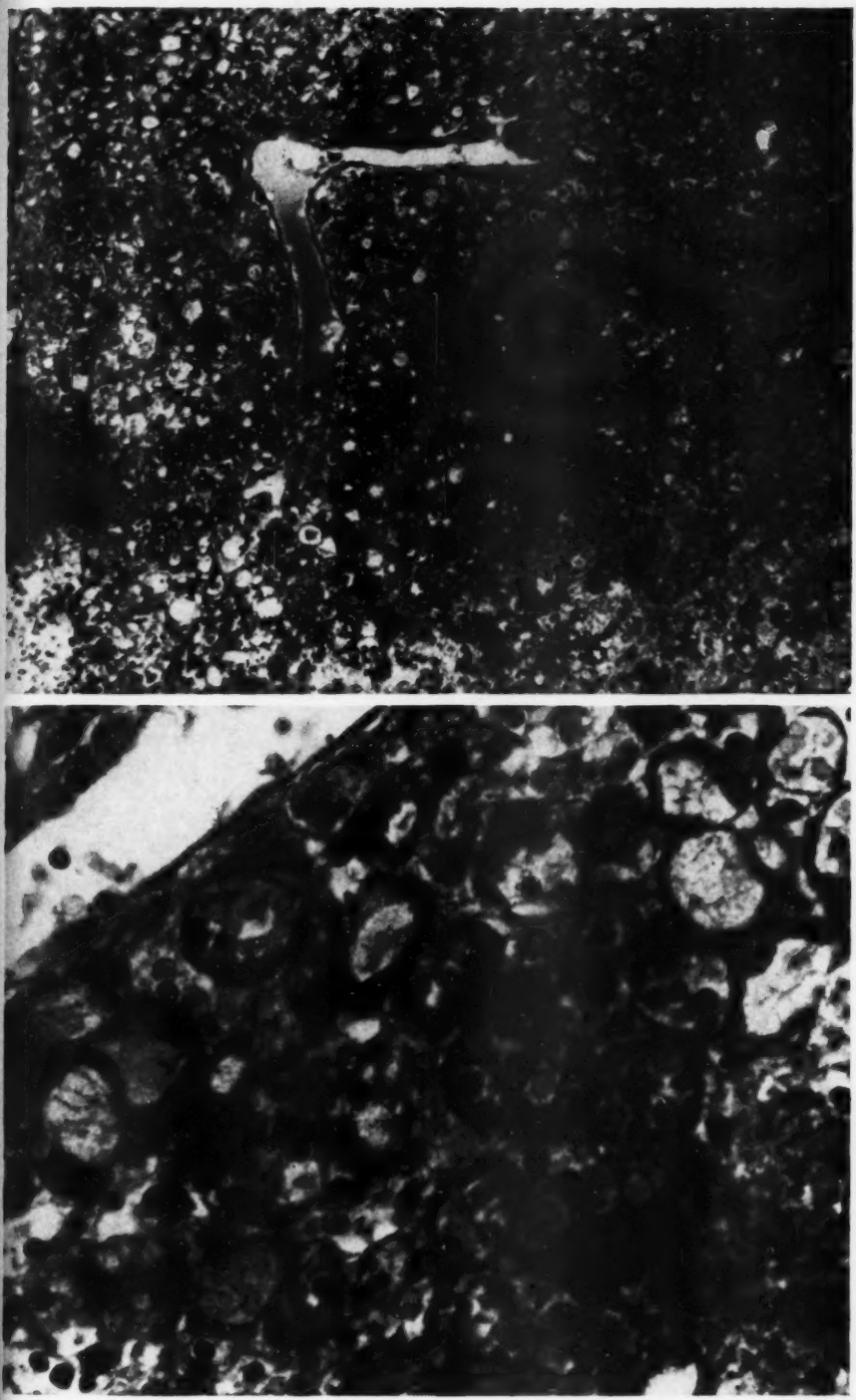
C

osin and Doljanski Rat's Liver in Allyl Formate Poisoning

PLATE 74

FIG. 7. From rat 17, which died 6 hours after the administration of allyl formate. Periportal area with extensive damages. The sinusoidal capillaries are broken up and the liver cells are swollen and contain large vacuoles filled with erythrocytes. × 170.

FIG. 8. Higher magnification of an area in Figure 7. × 800.



osin and Doljanski Rat's Liver in Allyl Formate Poisoning

PLATE 75

FIGS. 9-13. All from the same animal as Figure 7. Erythrocytes in liver cells. × 1600 to 1900.

Am

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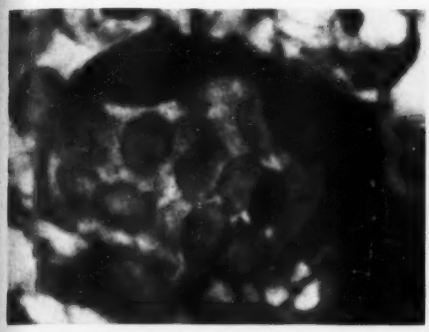

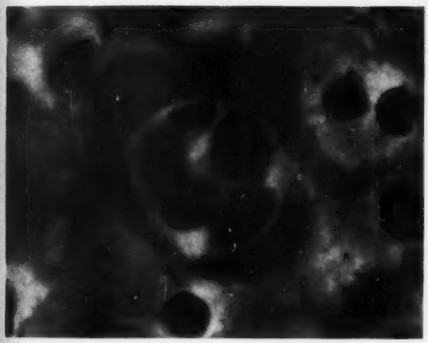

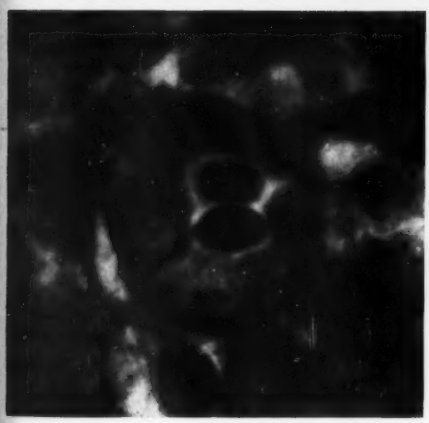

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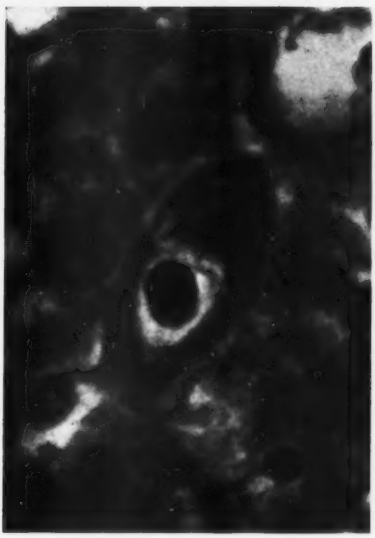

13

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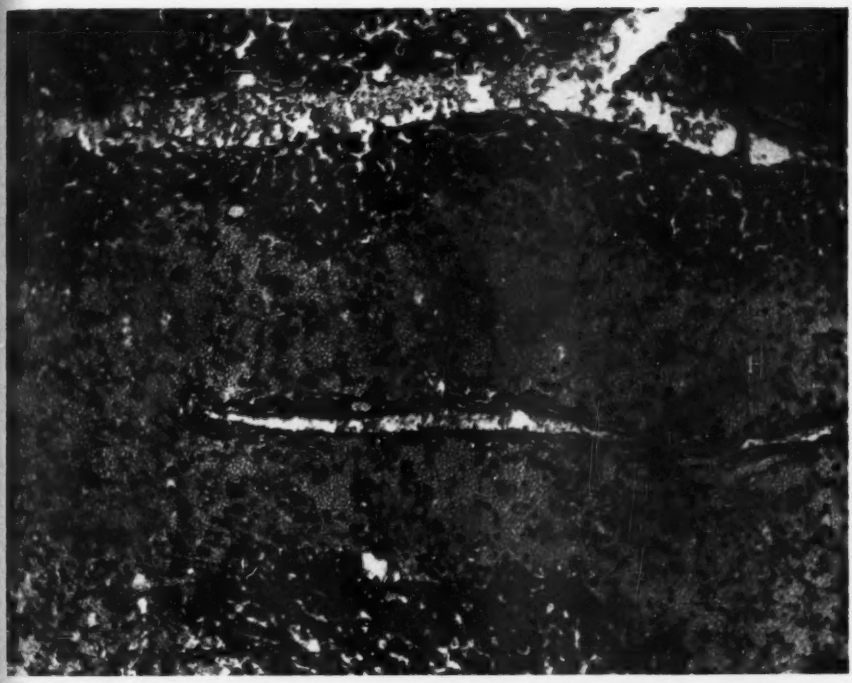
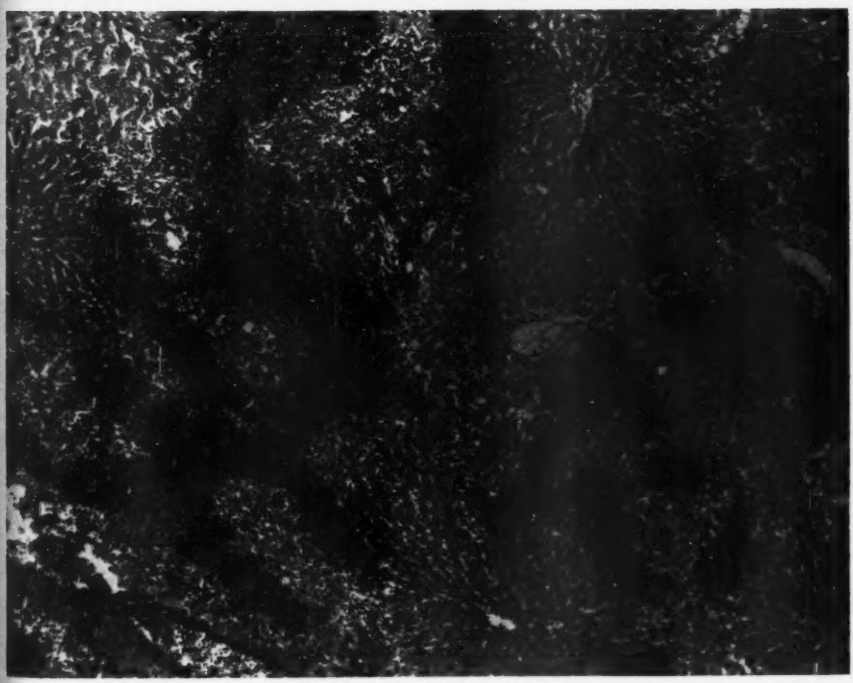
Rat's Liver in Allyl Formate Poisoning

PLATE 76

FIG. 14. From rat 20, which died 4 hours after the administration of a second dose of allyl formate. Extensive hemorrhages. × 169.

FIG. 15. From rat 18, which died 7 hours after the administration of a second dose of allyl formate. Zones of necrosis. × 72.





Rosin and Doljanski Rat's Liver in Allyl Formate Poisoning

PRIMARY TUMOR OF THE HEART CONTAINING EPITHELIUM-LIKE ELEMENTS *

W. A. D. ANDERSON, M.D.,† and EUGENE T. DMYTRYK, M.D.

(From the Department of Pathology, St. Louis University School of Medicine, St. Louis, Mo.)

Primary tumors of the heart, although rare, have excited such interest among those by whom they have been encountered that more than 100 cases have been reported. These cases have been collected and reviewed from time to time,[1-8] so no attempt will be made here to survey these reports completely.

In reviewing the descriptions of reported cases, one is struck by the fact that, with relatively rare exceptions, most of the primary cardiac tumors form a well defined group. Characteristic of this group of tumors are an almost constant origin from one atrium or the other and commonly from the interatrial septum, polypoid projection of the tumor into the cavity of the atrium, associated thrombosis, and a microscopic structure predominantly of myxomatous or angiomatous nature. Both benign and malignant forms occur. The terminology used in reporting these tumors is indicative of variable histologic structure and interpretation. The benign forms have been termed myxoma, fibromyxoma, hemangioma, hemangio-endothelioma, lymphangioma, lymphangio-endothelioma, pseudomyxoma, fibro-angiomyxoma, and hemangio-elastomyxoma. Malignant representatives that appear to fit in this group have been called myxosarcoma, fibromyxosarcoma, angiosarcoma, and angioreticulo-endothelioma (Kaposi's disease).

The majority of these tumors have been found in the right or left atrium, appearing to have originated from the atrial septum near the fossa ovalis, although other origins, as from the pulmonary artery,[6] have been described. The relative constancy of the site of origin of this group of tumors suggests that it has some significance. Ribbert [9] maintained that rests of embryonic mucoid tissue may persist in the heart, especially in the rim of the foramen ovale. Certainly the tendency to a particular site of origin is suggestive of derivation from some type of embryonic rest, in analogous fashion to the origin of certain other tumors.

It is not surprising that any tumor involving endocardium and projecting into the cardiac cavity should be associated with thrombosis. Debate has arisen, however, over the possibility that formation of the thrombus may be primary, with neoplasm developing in the thrombus.

* Received for publication, March 19, 1945.

† Now at Marquette University School of Medicine, Milwaukee 3, Wis.

Indeed, it has been maintained that some of the benign forms may not be of neoplastic nature, but simply thrombi which have undergone a myxomatous type of degeneration.[10] Warthin [11] described a mucoid fibroblastic tissue as a common result of organization of cardiac thrombi, and was unconvinced that a true myxoblastoma of the heart existed. However, it is not possible to doubt that truly neoplastic polypoid tumors containing a myxomatous tissue have been found in the heart. It may be, though, that the myxomatous element is a degenerative change in the stroma, or a result of a change in associated thrombotic material, rather than a truly neoplastic component. The arguments favoring the genuine neoplastic nature of at least some of these tumors have been considered in several papers [5,12] and need not be repeated here.

The following case appears to belong to this group of tumors, having the characteristic position and structure, with prominence of myxomatous change and abundant proliferation of small vascular channels. It is unique, however, in that included in it are cells which appear to be of epithelial or mesothelial nature, and which form gland-like cystic structures.

Report of Case

A. H., a white woman, 53 years of age, came to the hospital because of shortness of breath and swelling of the feet and legs. These symptoms had not been severe enough to interfere with her work until 4 weeks previously. No history of rheumatic fever was elicited. The findings were those of severe congestive cardiac failure, with auricular fibrillation and evidence of aortic regurgitation and stenosis. Symptoms increased in severity, and death occurred approximately 1 week after admission.

Post-mortem examination showed the anasarca and chronic congestive changes of viscera commonly associated with failure of the circulation. The unopened heart weighed 790 gm. The right atrium contributed largely to the increased size and weight of the heart, for it showed a bulky, rounded, firm enlargement to a diameter of 6 cm. The aortic valve was stenotic, with thickened, shortened, and adherent, calcified leaflets.

The distention of the right atrium was due to an egg-shaped tumor mass, measuring 8 by 5 cm., which filled and enlarged this portion of the heart. The upper and main portion of the tumor was an oval mass, 4 cm. in diameter, tightly enclosed on all except its lower end by the stretched wall of the atrium. This enclosing capsule of atrial wall was thinned out in its upper part to 1 mm. and even less in some regions. In some areas it was possible to strip away the tumor from the atrial wall along a line of cleavage, but in other parts, and particularly near the summit of the mass, the neoplastic tissue blended with the wall so that separation could not be accomplished without tearing.

Projecting downward from the main body of the tumor and hanging free above the tricuspid valve were some irregular nodular projections of the tumor mass. This free lower end was covered by a thin, smooth, grayish blue membrane which was continuous with the endothelial lining of the atrium. In some areas where this covering membrane was deficient, reddish brown nodules of friable material projected through the ulcerations. These were evidently masses of thrombotic material.

Sections through the tumor showed that the main bulk was composed of a firm, rubbery, highly translucent tissue of grayish color. This rubbery tissue was interrupted by some irregular white areas, more fibrous in appearance and consistency. Occasional small blood vessels, of a size just visible by macroscopic examination, and containing clotted blood, could be discerned.

In various parts of the tumor there was a honeycomb structure due to irregular, small, cyst-like spaces, measuring up to 1½ cm. in diameter, and filled with mucoid material of jelly-like consistency. Removal of this material showed that each cyst had a smooth, white, membrane-like lining. In other areas there was a more angiomatous appearance, with thin-walled spaces filled by dark-red clotted blood.

The lowermost portion of the tumor mass, particularly where the limiting membrane was deficient, was composed of brown and gray friable tissue, contrasting with most of the tumor in both color and consistency, evidently thrombotic material. This was separable from the rest of the tumor, and nowhere did the thrombus and the tumor tend to be firmly adherent or imperceptibly to merge, but rather there seemed to be a line of cleavage.

The microscopic structure of the tumor showed marked variation in different areas. Most of the tissue had a myxomatous appearance, being composed of relatively few cells set in an abundant, loose, pale-staining stroma. In some areas the stroma stained well with eosin, and had a homogeneous colloid-like appearance. In other regions the stroma was faintly basophilic or eosinophilic, and had a loose fibrillar structure most striking in appearance around the fairly numerous capillaries. Sparsely situated in this abundant stroma were a few small cells scantily arranged in short lines of cells to give a spider-like appearance.

Other areas were highly cellular, with scanty stroma, and an entirely different appearance. These highly cellular areas had a rather sarcomatous aspect, and were supplied by moderate numbers of thin-walled capillary vessels. Where these cellular areas bordered on myocardium, there was invasion between individual muscle cells and around bundles of the myocardial fibers, with associated degenerative changes and atrophy of the parenchymatous tissue. In some areas there was invasion through the muscle of the auricular wall and involvement of epi-

cardial fatty tissue. The component cells were small, with oval nuclei and scanty cytoplasm having indefinite outlines. Fairly numerous plasma cells and basophils were mixed with the tumor cells, particularly in the less cellular areas. Fibers stainable by silver impregnation were not associated with these cells. No transition from myocardial fibers was detectable nor could any evidence of striations be found. Mitotic figures were seen very infrequently.

In some small areas lymphocytes only were present, to the exclusion of other types of cells, with no evidence of follicle formation. Again, in other regions, small, thin-walled blood vessels were so abundant that the tissue had a hemangiomatous appearance. There were occasional areas of bone formation.

Glandular cyst-like spaces lined by epithelium-like cells were evident in various areas of the tumor. These varied from tiny gland-like structures to the larger cystic spaces which were evident grossly. The epithelium was usually in a single layer and varied from flat cells of endothelial appearance to tall columnar cells, closely placed, and with regular basal nuclei. In some areas the epithelial cells showed evidence of secretory activity. Some of the epithelial linings showed papillary infoldings, or nodular areas several cells in thickness. Amorphous material and desquamated cells formed the contents of the cystic spaces. The material in some of the cavities and within vacuoles in the lining cells stained with mucicarmine. Some of the lining cells had frayed inner margins suggestive of cilia, although nowhere was this definite enough to be unquestionable.

Deposition of pigment was common throughout various parts of the tumor. This was mostly in the form of brownish granules of hemosiderin pigment, which gave a Prussian-blue reaction. A curious finding was the staining of connective tissue trabeculae by bluish-black iron-containing pigment in a fashion similar to that seen in the spleen in sickle cell anemia. In several areas there were actual siderofibrotic nodules (Gandy-Gamna bodies), with bamboo-like segmented rods of yellowish and brownish color which simulated the mycelial threads of fungi.

Discussion

The position, the gross appearance, and the angiomatous and myxomatous microscopic character of this tumor clearly places it with the commonly described group of primary cardiac tumors. The unique feature lies in the inclusion of elements of epithelial appearance, which hitherto have not been described in such tumors. In position, the myxomatous and angiomatous portions of the tumor mass were in close association with thrombotic material, but had no constant relationship to

the epithelium-like structures. The appearance and relationships of the myxomatous and angiomatous elements give the impression that they are due to changes developing in a thrombus, possibly promoted by the mechanical forces brought into play by the peculiar position.

The explanation of the origin of the epithelium-like structures is not readily apparent. The presence of abundant channels and spaces lined by endothelium suggests the possibility of origin from such endothelium by a metaplastic change to cells having the appearance of epithelium. This possibility receives some support from the observation that in the lining of some of the cyst-like spaces a transition can be observed from flat cells resembling endothelium to tall columnar cells with basal nuclei. However, no evidence was found that any space having an epithelium-like lining was actually of vascular origin, from either content, structure of the wall, or connection with a definite blood or lymph channel. Vascular endothelium is not known to have potentialities for transition to tall columnar cells of epithelial character.

A more probable origin would seem to be from the inclusion of cell rests having potentialities for forming structures of this type. The striking constancy of origin of primary cardiac tumors of myxomatous or angiomatous nature from the region of the interatrial septum suggests the probability that they arise from some developmental abnormality or inclusion. Rezek [13] has described a tumor of microscopic proportions found beneath the endocardium of the right atrium and enclosed entirely within the interatrial septum. This tumor had a structure which linked it with the more bulky tumors which project into an atrial cavity, and it also contained elements of epithelial appearance. It may be, as he suggested, that such structures arise from epithelium displaced in the heart during early stages of embryonic development. As has been pointed out,[14] during early embryonic development there is an opportunity for heterotopic inclusion of entodermal tissue of the primitive foregut in the mesodermal tissue which forms the heart. The mucinous production in the tumor reported here would be consistent with such an entodermal origin. Some of the rare epithelial cysts of the heart [14-18] have been explained on this basis, and it would seem possible that a similar epithelial inclusion may have been the origin of the present tumor. A hypothesis of origin from a heterotopic inclusion is difficult to substantiate and equally difficult to refute. It is almost as easy to suggest that the heterotopic cell inclusion was of an even earlier stage and possessed potentialities for forming the epithelial and the mesenchymal structures evident in the tumor, and that hence it should be regarded as a mixed tumor or teratoma.

It must be considered as a possibility that this woman had a primary

carcinoma of the alimentary tract which remained small and could not be found by the usual autopsy procedures, but which nevertheless metastasized to the heart. For this there is lack of any suggestive evidence, and it may be dismissed as highly improbable.

Finally, the possibility of pericardial origin must be considered. Mesoblastic lining cells, such as those of the pleura and pericardium, have potentialities for forming epithelium-like structures [18] similar to those in the case described here, and serous epicardial cells have often been observed to differentiate into tall columnar cells.[14] These mesoblastic cells are capable of forming a mucoid substance. Some of the cystic structures found in the heart have been thought to have an epicardial origin, due to invagination of the outer part of the myocardial plate, which forms the epicardium, into the inner plate from which the myocardium is formed.[19] A pericardial origin seems to be favored by the marked variation in the form of the cells lining the cysts, transitions from low flattened cells to tall columnar cells being observable. However, extension of the tumor cells through the thin atrial wall with microscopic evidence of involvment of epicardium probably cannot be considered as signifying direct origin from pericardial tissue.

While inclined to feel that the evidence favors a pericardial origin for the epithelium-like structures of the tumor, we realize that considerations as to origin are speculative and inconclusive at present.

Conclusion

A primary myxomatous cardiac tumor arising from the right atrium is described. A unique feature of this tumor is the presence of epithelium-like cells forming gland-like and cystic spaces. The possible origins are discussed, and the conclusion reached that it is probably due to the inclusion of pericardial elements in the atrial wall during cardiac development. The myxomatous and angiomatous features of the tumor are believed to be the result of changes in associated thrombotic material.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 77

FIG. 1. External appearance of the heart, showing the bulge of the wall of the right atrium produced by the tumor.

FIG. 2. Cut surface of the tumor, showing the upper portion blending with the atrial wall, and the lower part free over the valve. Cystic areas are evident on the cut surface.

FIG. 3. Myxomatous area of the tumor. × 150.

FIG. 4. Region of the tumor showing a portion of the lining of two cystic spaces, other gland-like structures, and cellular infiltration around remnants of muscle fibers. × 120.

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Anderson and Dmytryk Primary Tumor of the Heart

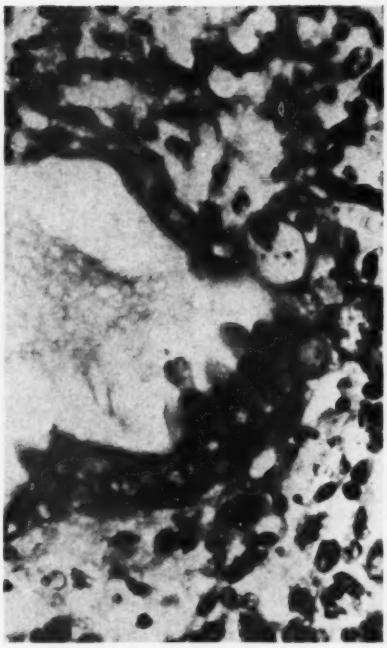
PLATE 78

FIG. 5. A region of the tumor showing several gland-like spaces and cystic areas. × 300.

FIG. 6. A portion of the wall of one of the cysts, having the appearance of secretory activity. × 550.

FIG. 7. A portion of the lining of one of the larger cysts, showing columnar cells with frayed inner margins. × 750.

FIG. 8. An area of a cyst wall in the tumor, showing columnar lining cells with basal nuclei. × 300.



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Anderson and Dmytryk Primary Tumor of the Heart

PLATE 79

FIG. 9. Bone and marrow formation in the tumor, and some epithelium-like elements. × 120.

FIG. 10. Remnants of myocardial fibers surrounded by the small, dark cells which composed most of the tumor. × 550.

FIG. 11. An area of pigment accumulation, showing impregnation of fibers. × 150.

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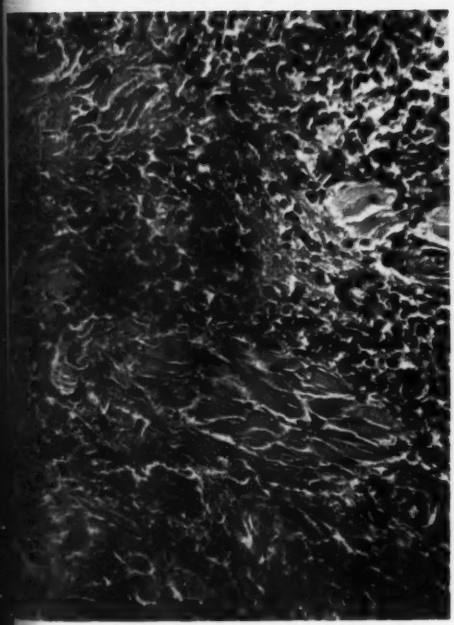

11

GROSS VASCULARITY OF THE MITRAL VALVE AS A STIGMA OF RHEUMATIC HEART DISEASE *

SIMON KOLETSKY, M.D.

(From the Institute of Pathology, Western Reserve University, School of Medicine, Cleveland 6, Ohio)

Grossly vascularized mitral valves are usually the seat of rheumatic endocarditis, *i.e.*, mitral stenosis or nondeforming valvular disease. Sometimes, however, the vascularized leaflets appear normal in the gross and no conclusive rheumatic stigmas are present elsewhere in the heart. This study deals with the morphology of vascularized mitral valves in hearts with and without gross rheumatic disease.

METHOD

Selection of hearts was based on diffuse vascularity of the anterior mitral leaflet, extending from attachment to line of closure. Vascularity of the posterior mitral leaflet, although present in many instances, was not as well marked and was often focal and of variable pattern. Hearts with vascularization limited in the gross to the basal third or half of the anterior leaflet were excluded.

The study comprised 150 hearts with grossly vascularized anterior mitral leaflets, divided into three groups as follows: *Group 1*, 50 hearts with no conclusive gross rheumatic disease; *Group 2*, 50 hearts with nondeforming rheumatic mitral disease; *Group 3*, 50 hearts with mitral stenosis.

In group 1 no gross stigmas of rheumatic disease were present in the mitral valve or elsewhere in the heart. In the hearts of group 2 the mitral valve was the seat of nondeforming valvulitis, *i.e.*, opacity and thickening of the leaflets especially in the line of closure, without shortening or commissural fusion, and with thickening or fusion of chordae tendineae. The hearts in groups 2 and 3, especially those with mitral stenosis, frequently showed deforming or nondeforming disease of aortic, tricuspid, or pulmonic valves and, less commonly, chronic endocarditis of the left atrium and pericardial adhesion.

Fifty nonrheumatic adult hearts with grossly avascular valves were included as a control.

Microscopic sections, prepared from all hearts according to the method of Gross, Antopol, and Sacks,[1] were stained with hematoxylin and eosin. Duplicate sections were stained with the combined Weigert and van Gieson methods for elastic and connective tissue. The mitral leaflets were examined for type, location, and distribution of vessels,

* Received for publication, April 24, 1945.

cellular exudate, and endocardial reduplications. Five other cardiac sites were examined for rheumatic stigmas, *i.e.*, the posterior papillary muscle of the left ventricle, the posterior endocardial wall of the left atrium, and the aortic, tricuspid, and pulmonic valves.

The lesions accepted as rheumatic were: *myocardium,* Aschoff nodules; *left atrial endocardium,* Aschoff nodules, subendothelial plaques, vascularity and cellular exudate of the endocardium proper, and hypervascularity and cellular exudate of the subendocardium; *valves,* vascularity of the free portions, especially with vessels of arterial type, cellular exudate, and endocardial reduplications. Atrial and valvular stigmas frequently occurred in combination. Equivocal changes were not included as stigmas.

Description of Rheumatic Lesions

Three characteristic microscopic stigmas of rheumatic heart disease are: (1) vascularization of valves with thick-walled arteries, (2) Aschoff nodules, and (3) endocardial reduplications of valves, especially mitral and aortic. A description of the first is given subsequently.

Aschoff nodules consist of typical Aschoff cells, the Anitschkow myocytes, with "owl-eyed," fibrocytoid, or pyknotic nuclei contained in a collagenous matrix which is swollen and coarsely granular. The cytoplasm of the cells is frequently basophilic and has a ragged, poorly defined peripheral border. Only typical nodules of this sort were accepted as rheumatic.

Endocardial reduplications occur in a subendothelial location, affecting the auricularis and ventricularis layers of atrioventricular valves, and the arterialis and ventricularis layers of semilunar valves. Gross and Friedberg [2] attributed this change to proliferation of fixed mesenchymal cells which are then transformed into connective tissue with subsequent elastification. The lesion appears to consist of an additional layer of fibro-elastic tissue superimposed on the endocardial surface of the valve. The reduplications are sometimes confined to the attachment or free portion of the valve, but may be diffuse, extending from the base of the leaflet well out toward the line of closure. The valvular attachment is a common site. Here, involvement of the ventricularis or arterialis layer of the aortic valve produces the so-called subaortic angle or pocket lesion respectively, while involvement of the ventricularis layer of the mitral valve results in the subvalvular pocket lesion. Reduplications may be vascularized, especially with thick-walled small arteries, may occasionally contain smooth muscle cells, and sometimes show cellular exudate, scarring, or calcific deposit. Although generally com-

posed of only one layer of connective tissue, there are reduplications of multiple type which consist of two or more distinct layers separated by a dense elastic membrane. Rheumatic reduplications are occasionally difficult to distinguish from similar changes in nonrheumatic hearts. Gross and Friedberg [2] pointed out that since collagenous thickening and elastification of valvular layers usually occur normally with increasing age, reduplications should not be attributed to rheumatic disease unless they are beyond the normal range or show qualitative differences such as vascularity or cellular exudate.

Morphology of the Vessels in Vascularized Mitral Valves

Gross Description. A fairly detailed account of the gross appearance of vascularized valves was given by Ribbert.[3] In the present study the vascularization of mitral leaflets was generally similar in the hearts of groups 1 and 2. In mitral valves, the seat of stenosis, the vascularity was often obscured because of thickening and opacity of leaflets.

The vessels extended from the valvular attachment to the line of closure and varied from few to an extensive branching network with numerous anastomoses. Variation in pattern was much more frequent in the posterior than in the anterior leaflet. In the latter there were often one or two main arterial stems at the base of the leaflet, occasionally more than two, arising in the lateral thirds, especially adjacent to the posterior commissure, and less commonly in the central third of the base (Figs. 1 to 4).

The vessels were delicate in appearance or formed prominent cord-like structures situated just beneath or projecting slightly upon the endothelial surface of the leaflet on the atrial side. They were usually readily visible to the naked eye, especially when filled with blood. Sometimes they were easier to visualize when the valve was viewed obliquely or by transmitted light.

Microscopic Examination. The structure of the vessels in both leaflets of the mitral valve was generally similar in practically all hearts of groups 1, 2, and 3. The vessels were sectioned in longitudinal, transverse, and oblique planes.

The leaflets contained capillaries, arterioles, and arteries; veins were probably present but were difficult to distinguish from the arteries. The vessels were situated on the atrial side of the valve, were frequently subendothelial, and might project upon the surface of the valve. In the anterior leaflet they occurred mainly in the auricularis layer; involvement of the spongiosa was less conspicuous and of fibrosa and ventricularis, rare. In the posterior leaflets the vessels were situated

in the spongiosa and auricularis layers and occasionally in the fibrosa and ventricularis. At the line of closure and tip of the valve the vascularity was often abundant and diffuse.

The predominant vessel was a small artery with thick musculo-elastic wall (Figs. 5, 6, and 7). Although this usually measured 150 to 250 μ in transverse diameter, there was a wide range from about 50 to 500 μ. Most vessels of arteriolar size, *i.e.*, less than 75 μ in diameter, conformed to this type (Fig. 8). Aside from capillaries and a small number of arterioles, there were very few, if any, vessels of normal appearance.

The structure of the musculo-elastic artery differed substantially from the normal small artery. The vessel was composed principally of smooth muscle cells orientated longitudinally instead of in the customary transverse manner, and partly separated by elastic fibers (Fig. 9). On transverse section the cytoplasm of the muscle cells was often clear or vacuolated so that the vessel wall had a honeycombed appearance. The lumen was generally narrow, might be eccentric, and was lined by endothelium which appeared to rest directly on muscle. A thin circular layer of muscle, a few cells in thickness, was frequently present at the periphery of the vessel, but this was rarely well developed. A clear-cut adventitia was not discernible.

Some vessels conformed to the so-called intimal musculo-elastic artery described by Gross, Kugel, and Epstein [4] as characteristic of rheumatic heart disease (Fig. 10). Here, as shown most clearly by section in the long axis of the vessel, the longitudinal muscle was confined to the region of the intima and was surrounded by a transversely arranged media. However, in most musculo-elastic vessels it was difficult to identify intimal and medial layers, either in longitudinal or transverse section, because of the variable disposition of the elastica.

A clear-cut internal elastic lamina was usually absent. Sometimes a fairly well defined, circular, elastic membrane, single or reduplicated, was present, but this varied in location, *i.e.*, adjacent to endothelium, or at the outermost margin of the vessel, or between these sites. Accordingly, the longitudinal muscle cells lay entirely within, or external to, or on both sides of this elastica. Occasionally there were two separate elastic laminae, usually situated near the endothelium and at the outer border of the vessel respectively. The musculo-elastic vessels often resembled the *état reticulaire* of Rabé [5] but the latter is a distinct lesion of the medial layer resulting from degeneration and vacuolization of muscle cells.

The elastic component of the arteries often underwent progressive change in amount and configuration. The original network of delicate

fibers was condensed and rearranged to form single or reduplicated circular laminae; then, through thickening and further reduplication, the elastica formed concentric bands extending through the entire wall (Fig. 11). Associated with this process were deposition of collagen, and atrophy of muscle cells with connective tissue replacement. The end-result was an artery of fibro-elastic type. The time relations of these progressive changes are not clearly known. It has been suggested that fibro-elastic metamorphosis may occur within 1 year.[2]

A summary of the findings is given in Tables I to IV.

TABLE I

Distribution of Musculo-Elastic Vessels in 150 Hearts with Grossly Vascularized Mitral Valve

	Number of cases	Anterior mitral leaflet	Posterior mitral leaflet	Posterior papillary muscle of left ventricle	Left atrium	Aortic valve	Tricuspid valve	Pulmonary valve
Group 1: Hearts with no conclusive gross rheumatic disease	50	44	38	0	2	6	2	0
Group 2: Hearts with nondeforming rheumatic mitral disease	50	48	46	1	1	11	5	1
Group 3: Hearts with mitral stenosis	50	48	44	1	4	25	16	6
Totals	150	140	128	2	7	42	23	7

Vascularity of the Mitral Valve. The anterior mitral leaflets of all hearts in groups 1, 2, and 3 were vascularized diffusely, usually to the line of closure or tip; this leaflet contained vessels of musculo-elastic type in 44, 48, and 48 hearts respectively in the three groups (Table I). The posterior mitral leaflet was vascularized in 46, 49, and 48 hearts and musculo-elastic vessels were found in 38, 46, and 44 hearts respectively in groups 1, 2, and 3 (Table I). In the cases of mitral stenosis or nondeforming mitral disease, vascularity of this leaflet generally extended to the line of closure, although in a few instances the vessels were apparently confined to the basal third or half. Of the 46 vascularized leaflets in hearts with no gross rheumatic disease, the vessels were confined to the basal third or half in 20 cases and extended beyond the basal half, usually to the line of closure, in 26 cases.

Cellular Exudate of the Mitral Valve. In 26, 36, and 41 hearts respectively of groups 1, 2, and 3 (Table II) cellular exudate of the

mitral valve was present. The lesion generally involved both mitral leaflets but in a few instances it was confined to one. The cells were principally lymphocytes, their number small, and the distribution focal. However, in several hearts with mitral stenosis the exudate was abundant and diffuse.

Endocardial Reduplications of the Mitral Valve. Endocardial reduplications of the mitral valve were observed in 23 mitral valves of group 1, 28 of group 2, and 40 of group 3 (Table II). In the hearts with mitral stenosis, the reduplications were well developed, often multiple and vascularized, and contributed to the thickness of the leaflets. In groups 1 and 2, however, the lesions were less well developed and were frequently excluded because they were equivocal; the

TABLE II

Microscopic Lesions of Mitral Leaflets in 150 Hearts with Grossly Vascularized Mitral Valve

	Number of cases	Endocardial reduplications	Cellular exudate
Group 1: Hearts with no conclusive gross rheumatic disease	50	23	26
Group 2: Hearts with nondeforming rheumatic mitral disease	50	28	36
Group 3: Hearts with mitral stenosis	50	40	41
Totals	150	91	103

changes generally involved the attachment and the basal portion of posterior leaflets and were uncommon in anterior leaflets.

Stigmas of Rheumatic Disease. Table III gives the number of hearts showing rheumatic stigmas at each of five sites, namely, the posterior papillary muscle of the left ventricle, the left atrium, and the aortic, tricuspid, and pulmonic valves. The aortic and tricuspid valves and the left atrium were involved most frequently. The progressively higher incidence of positive stigmas in each of the three groups is probably related to the increasing severity of the rheumatic disease.

Musculo-elastic arteries occurred much more commonly in the mitral valve than elsewhere in the heart. The incidence was slightly greater in the anterior than in the posterior leaflet. Aside from the mitral valve, the most frequent sites were aortic and tricuspid valves. Involvement of the pulmonic valve, left atrium, and posterior papillary muscle of the left ventricle is uncommon or rare (Table I). The ves-

sels were occasionally observed in the myo-epicardial wedge adjacent to the valve rings.

Table IV gives the distribution of rheumatic lesions according to the number of sites involved. Involvement of three or more sites occurred in only 7 hearts of group 1, as compared to 14 in group 2 and 33 in group 3. At least one site was involved in 38, 42, and 49 hearts respectively in groups 1, 2, and 3; conversely, all five locations were

TABLE III

Distribution of Microscopic Stigmas of Rheumatic Disease in 150 Hearts with Grossly Vascularized Mitral Valve

	Number of cases	Posterior papillary muscle of left ventricle	Left atrium	Aortic valve	Tricuspid valve	Pulmonary valve
Group 1: Hearts with no conclusive gross rheumatic disease	50	6	8	22	14	12
Group 2: Hearts with nondeforming rheumatic mitral disease	50	8	15	36	19	12
Group 3 Hearts with mitral stenosis	50	12	37	40	34	23
Totals	150	26	60	98	67	47

negative in 12 hearts of group 1, 8 hearts of group 2, and only 1 heart in group 3.

In 38 of the 50 nonrheumatic hearts the free portions of all valves were avascular; in 10 hearts one or more valves showed capillaries and an occasional arteriole just beyond the attachment or in the basal third of the free portion, and this was associated in 9 of the hearts with projections of the annulus fibrosis or of auricular myocardium; in 2

TABLE IV

Distribution of Rheumatic Stigmas According to Number of Sites Involved in 150 Hearts with Grossly Vascularized Mitral Valve

	Number of cases	All sites negative	One site involved	Two sites involved	Three or more sites involved
Group 1: Hearts with no conclusive gross rheumatic disease	50	12	23	8	7
Group 2: Hearts with nondeforming rheumatic mitral disease	50	8	14	14	14
Group 3: Hearts with mitral stenosis	50	1	9	7	33

hearts there was a moderate number of vessels, including musculo-elastic arteries, in the basal third of the mitral valve. The last were considered probable instances of rheumatic endocarditis. Cellular exudate and well developed endocardial reduplications were absent in all valves.

Comment

Hearts with diffuse gross vascularity of the anterior mitral leaflet show essentially similar microscopic lesions regardless of whether or not gross rheumatic disease is present. These lesions are identified by the type, location, and distribution of vessels in the mitral valve, cellular exudate and endocardial reduplications of the mitral valve, and the presence of rheumatic stigmas at other cardiac sites. The differences between hearts with no conclusive gross rheumatic disease and those with nondeforming mitral disease or mitral stenosis are quantitative rather than qualitative.

Hypervascularity of valves, especially with arterial vessels of musculo-elastic type and with deep penetration to the line of closure, is characteristic of rheumatic disease. Similar hypervascularity is also observed in rheumatic endocarditis of the left atrium.[6] The formation of new blood vessels occurs in the acute phase of rheumatic inflammation and the vessels remain as a permanent stigma when activity ceases. Gross vascularity is much more frequent in the mitral valve than in the aortic, tricuspid, or pulmonic valves.

The extent of vascularity is not necessarily proportional to the degree of valvular deformity. Some mitral valves with nondeforming rheumatic disease are more richly vascularized than are others which have become the seat of stenosis. Similarly, stenotic aortic valves are sometimes sparsely vascular in comparison with mitral or tricuspid valves which show nondeforming lesions. Moreover, while vascularity is probably the most common stigma of rheumatic endocarditis, it is not constant. Occasionally, valves with nondeforming disease and rarely even deformed valves are apparently avascular.

The new blood vessels are presumably derived from pre-existing ones situated in the atrial myocardial wedge at the attachment of the leaflets. Harper [7] has described the development of capillaries in acute rheumatic endocarditis by proliferating endothelial buds which acquire an adventitia from connective tissue elements within the valve. Possibly the musculo-elastic arteries are also derived by conversion of connective tissue into muscle. An alternative origin, *i.e.*, from muscle tissue already present in the valve,[8] is suggested by the peculiar structure of these vessels and their frequency in the mitral valve. Morphologically, the arteries resemble closely the longitudinal smooth muscle bundles

normally found in the mitral leaflets, principally in the auricularis layer where they are distributed well out toward the line of closure. In rheumatic inflammation these bundles might undergo transformation into vessels either by forming a wall around proliferating capillaries or possibly by the development of endothelium-lined channels within the muscle. Longitudinal muscle bundles are uncommon in the free portion of the normal aortic valve but are sometimes present at the attachment, especially in the subaortic angle.

The nature of the stimulus responsible for the formation of vessels is not clear. Ribbert [3] assumed that chemotactic substances produced by endocarditis are reabsorbed and stimulate proliferation of the endothelium of normal vessels. He pointed out that a similar process occurs in inflammatory lesions of the cornea. Harper [7] suggested toxic or bacterial injury to endothelium and cellular elements of the cusps. Regardless of mechanism, the evidence indicates clearly that rheumatic fever is the principal underlying cause.

Musculo-elastic arteries are observed in valves which are the seat of acute rheumatic endocarditis. Although essentially similar in structure to the chronic rheumatic lesion, the vessels usually show edema, swelling and variation of polarity of nuclei, and a minimal amount of elastica. Gross and Friedberg [2] claimed that a period of at least 6 weeks is required for development, a conclusion based on the absence of these vessels in 20 of 21 patients dead within 6 weeks of the first rheumatic attack. My material did not permit investigation of this point. However, the arteries were uniformly present at autopsy in the mitral valves of 15 children with acute rheumatic heart disease. Fourteen gave a history of at least one previous episode of acute rheumatic fever from several months to 5 years prior to death. Only one child had a single fatal attack and this was of 4 months' duration.

The presence in valves of musculo-elastic arteries is perhaps pathognomonic of rheumatic disease. Vessels of this type are sometimes observed in cardiovascular syphilis, but the latter is generally confined in the heart to the aortic valve where a distinction between syphilitic and rheumatic disease can usually be made without difficulty.[9,10] In other etiologic forms of heart disease such as those which are hypertensive and arteriosclerotic, and in cor pulmonale, valvular lesions are not produced. Vessels occur in valves with endocarditis lenta but there is commonly an underlying rheumatic basis. Gross [11] found that the blood vessels in the valves of 27 hearts with atypical verrucous endocarditis consisted essentially of capillaries of the granulation-tissue, endothelial-bud type; vessels with muscular walls were rarely observed except in hearts considered to have been the seat of preceding rheumatic infection. A study of 8 hearts with atypical verrucous endo-

carditis in my own material was confirmatory. At present there is no evidence that other infectious diseases, such as scarlet fever, streptococcal pharyngitis, or pneumonia, may induce formation of blood vessels in heart valves.

A control study was made of the blood vessels in such lesions as pleural and pericardial scars, arteries with canalized thrombi, and chronic inflammatory processes with the formation of granulation tissue. Of a total of 100 such cases, about one-third showed small, thick-walled arteries with a muscle component. The latter revealed a variable arrangement, either transverse or longitudinal. Musculo-elastic arteries, usually of intimal type, were found only occasionally in contrast to the frequency and predominance of these vessels in rheumatic valves (Fig. 12).

Whether human valves may normally be vascularized is still controversial. By the paraffin section method, most grossly normal valves are entirely avascular; some show capillaries and perhaps arterioles limited to the basal third of the valve where the vessels are usually contained within projections of the myocardium or of the annulus fibrosis into the free part of the leaflets.[12,13] Dow and Harper [14] reported similar results in injected hearts. Recently, however, Wearn and Moritz,[15] also using the technic of injection, claimed that 32 per cent of a noninflammatory group of 255 hearts showed vascularity beyond the basal third in one or more leaflets or cusps.

From the morphologic standpoint the issue is not whether normal valves are vascularized, but whether vascularized valves are normal, *i.e.*, show no evidence of endocarditis, chronic or healed. The latter can often be established clearly by means of microscopic stigmas of inflammation. Difficulty arises in borderline cases, especially those of extinct disease in which endocarditis may heal without leaving recognizable stigmas other than the vessels. In such instances, a decision as to whether the vascularity is normal or the result of inflammation may not be possible. However, the structure of the vessels alone will often provide evidence for an inflammatory basis. The presence of musculo-elastic arteries is strongly indicative of previous endocarditis. Since these vessels are in all probability acquired, valves containing them cannot be considered normal.

Summary and Conclusions

Hearts with diffuse gross vascularity of the mitral valve almost uniformly show microscopic stigmas of inflammatory disease, rheumatic in origin. Hence such vascularity can be regarded *per se* as an acceptable gross stigma of rheumatic heart disease.

The principal vessels in vascularized mitral leaflets are small, thick-

walled arteries or arterioles of musculo-elastic type. These consist mainly of longitudinally disposed smooth muscle cells separated by elastic fibers. In the mitral valve such vessels are characteristic, and probably pathognomonic, of rheumatic fever.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 80

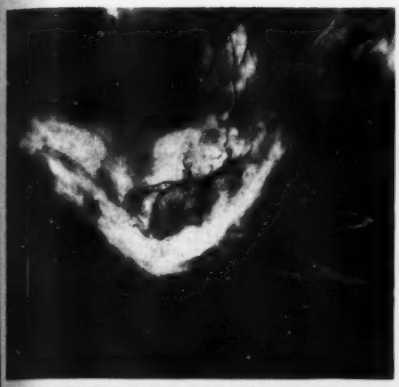
FIG. 1. Vascularized anterior mitral leaflet in a white male, 60 years old, with no gross rheumatic heart disease. There is one main arterial stem at the base of the leaflet near the posterior commissure.

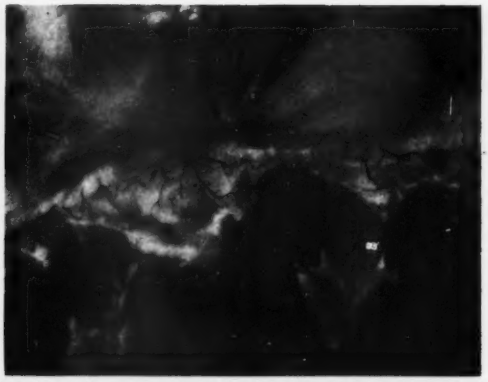
FIG. 2. Diffusely vascularized mitral valve in a white male, 61 years of age, with no gross rheumatic heart disease. The vessels extend to the line of closure.

FIG. 3. Vascularized anterior mitral leaflet in a white male, 65 years old, with non-deforming rheumatic heart disease, as viewed by transmitted light. There are three main arterial stems at the base of the leaflet.

FIG. 4. Vascularized anterior mitral leaflet in a white male, 21 years old, with no gross rheumatic heart disease, as viewed by transmitted light. Two main arterial stems are present at the base of the leaflet.

FIG. 5. Transverse section of a musculo-elastic artery, 168 μ in diameter, in an anterior mitral leaflet. The vessel wall has a honeycombed appearance. Hematoxylin and eosin stain. $\times$ 415.





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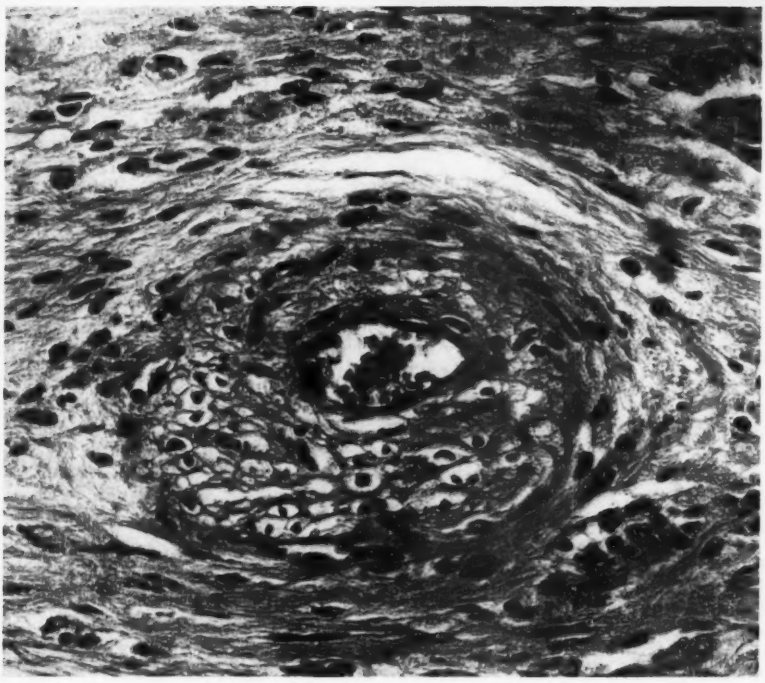

5

Koletsky Vascularity of Mitral Valve

PLATE 81

FIG. 6. Same vessel as shown in Figure 5. There is a dense elastic band at the periphery of the artery. Elastica-van Gieson stain. × 330.

FIG. 7. Transverse section of a musculo-elastic artery, 168 μ in diameter, in an anterior mitral leaflet, showing honeycombed appearance of the wall. A prominent elastic lamina is present near the endothelium. Elastica-van Gieson stain. × 330.

FIG. 8. Musculo-elastic vessel of arteriolar size, 70 μ in diameter, in an anterior mitral leaflet. Elastica-van Gieson stain. × 330.

FIG. 9. Longitudinal section of a musculo-elastic artery, 112 μ in diameter, in an anterior mitral leaflet. The muscle fibers are arranged in the long axis of the vessel. Hematoxylin and eosin stain. × 330.

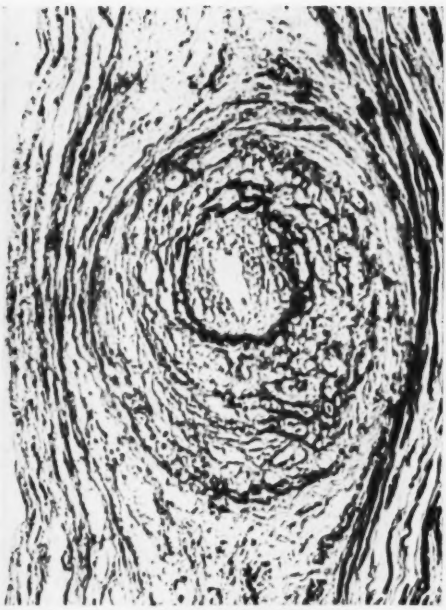

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PLATE 82

FIG. 10. Artery of intimal musculo-elastic type, 90 μ in diameter, in an anterior mitral leaflet. Elastica-van Gieson stain. $\times$ 415.

FIG. 11. Musculo-elastic artery, 140 μ in diameter, showing the fibro-elastic stage. Concentric elastic bands extend through the entire wall of the vessel. Elastica-van Gieson stain. $\times$ 330.

FIG. 12. Artery of intimal musculo-elastic type, 100 μ in diameter, in pericardial scar. Hematoxylin and eosin stain. $\times$ 330.

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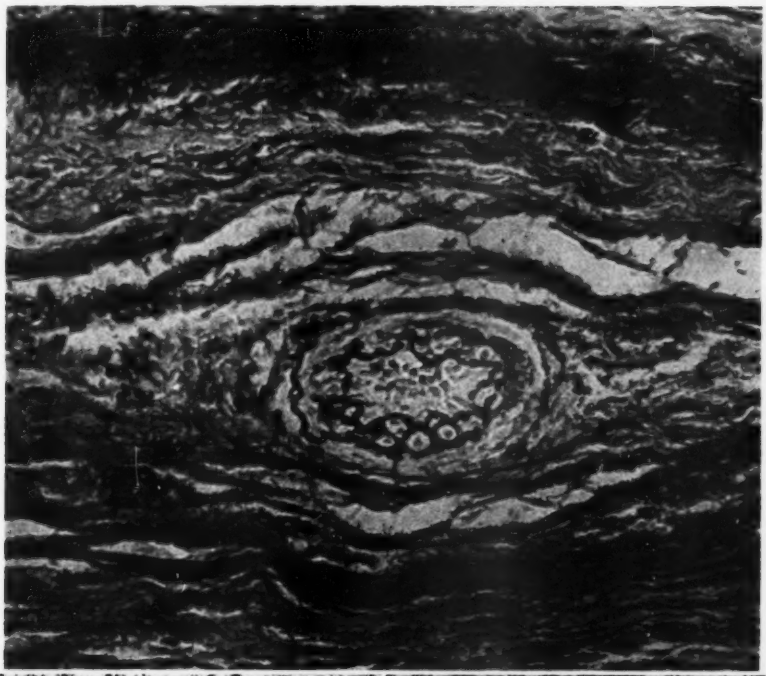

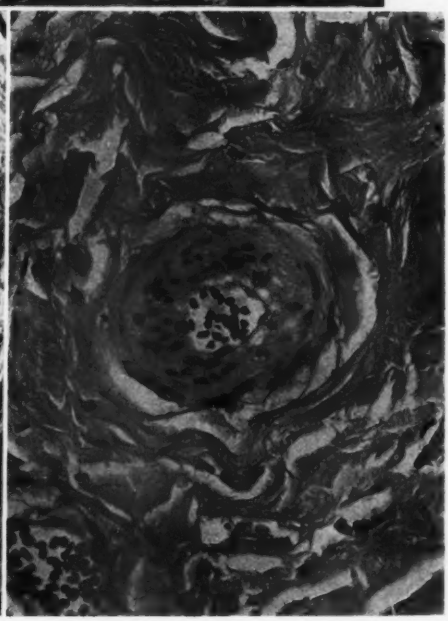

12

Koletsky Vascularity of Mitral Valve

OBSERVATIONS ON THE PATHOLOGICAL CHANGES PRODUCED BY A TOXIC SUBSTANCE PRESENT IN BLUE-GREEN ALGAE (MICROCYSTIS AERUGINOSA)*

C. T. Ashworth, M.D., and M. F. Mason, Ph.D.

(From the Departments of Pathology and Experimental Medicine, Southwestern Medical College, and Parkland Hospital, Dallas, Texas)

The presence of a toxic substance or substances in certain species of blue-green algae has been the subject of a number of reports.[1-3] In the case of one species, *Microcystis aeruginosa,* somewhat more extensive observations have been made upon the pharmacological and chemical properties of the toxic component.[3,4] Extracts prepared from *M. aeruginosa,* when injected subcutaneously, intraperitoneally, or intravenously, produce a characteristic response in several species of laboratory animals. A variable latent period is followed by pallor, decline in blood pressure, tachycardia, hypothermia, and death consequent to respiratory failure in a length of time dependent upon the kind of animal employed and the dose administered.[4] Upon autopsy, the liver is found uniformly to be dark reddish blue, and engorged, bleeding profusely after incision.

We are unaware of any detailed studies of the pathological changes produced by the toxic agent in *M. aeruginosa.* Thus it is the purpose of this communication to describe the structural alterations in such organs of rats as are affected following the administration of a maximal sublethal dose of algal extract.

Method of Study

Albino rats weighing about 200 gm. were employed in all experiments. The animals were maintained on a standard colony diet. The extract employed was prepared by shaking dry algal powder (which had previously been extracted with anhydrous chloroform, acetone, and ether) with about fifteen times its weight in water for three successive times. The aqueous phases were separated by prolonged centrifugation, pooled, and transferred to a series of stoppered serological tubes and stored in the frozen state at low temperature. The toxicity of the extract thus prepared and stored appears to be maintained indefinitely. The proportions employed yielded an extract for which the maximal sublethal dose was approximately 1 cc. per 100 gm. of rat.

The extract was administered intraperitoneally to a series of rats, and these were sacrificed at intervals following the injections. Portions of liver, brain, heart, lungs, spleen, kidneys, and gastrointestinal tract

* Received for publication, April 10, 1945.

were fixed in 10 per cent formalin. Sections were cut from paraffin and stained with hematoxylin and eosin. Sudan IV was used for staining frozen sections for fat in liver, kidney, and myocardium.

Results

Gross Observations

Fifteen to 30 minutes after the injection the liver was slightly enlarged, tense, and redder than normal. The centers of the lobules showed increased blood content. There were no gross changes noted in other organs at this early stage.

The livers were markedly enlarged in animals examined 3 to 6 hours after injection of the extract, weighing about 25 per cent more than those of normal control rats. This increased weight was not accounted for by blood content of the large veins since the amount of blood which could be drained from the veins was approximately the same by weight in the poisoned animals as in the control group. There was an increased blood content in the parenchyma of the liver, however, as indicated by the bloody fluid which dripped in excess from the cut surface and by the engorgement of the central portions of the lobules. The latter feature was responsible for enlargement of the lobule as a whole. The remaining periphery of the lobules was grayish red. The liver tissue was softened and friable. In some instances the parenchyma was partially liquefied so that a thick, creamy, dark bluish red fluid could be pressed from the cut surface. There was usually a diffuse alteration so that all lobes of the liver presented the appearance described. However, in the early stages there was occasionally patchy involvement so that some lobes or portions of lobes retained an essentially normal gross structure. The hilar portions of the lobes appeared to be the regions first involved.

There was no detectable change in the weights of the heart, lungs, spleen, or kidneys in these acutely poisoned animals. The lungs were hyperemic in animals dying spontaneously but usually were normal in color and air content in animals which were sacrificed. A few rats exhibited small hemorrhages in the lung tissue. The kidneys appeared slightly hyperemic, but otherwise seemed normal.

Two to 3 days after a maximal sublethal dose, the liver had undergone remarkable changes. It was shrunken to about two-thirds its normal size and was dull yellow. It was soft and had a mottled appearance. This mottling was due to red areas marking the slightly enlarged central veins, with surrounding yellow parenchyma. The blood content of these yellow livers was not increased. The other tissues were slightly

icteric. There was no excess fluid in the serous cavities. The kidneys had a brown tinge, were slightly softened and larger than normal. The urine was dark yellow. The lungs were normal at this stage except for occasional petechial hemorrhages. The blood clotted very slowly, sometimes requiring 10 or 15 minutes. The animals had lost a great deal of weight and exhibited marked general pallor which showed, also, in the conjunctiva.

Five days after the maximal sublethal dose the liver had returned to an almost normal state. It still had a yellow tinge but the mottled appearance had disappeared and other organs were, at this time, normal in appearance. The animals still appeared cachectic, pale, and anemic. One month after the injection no gross changes in the liver or other organs could be discerned, and the animals appeared to be entirely normal.

Histological Observations

Liver. As early as 15 minutes after intraperitoneal injection the parenchymal cells were found to be swollen and their outlines less prominent (Fig. 1). The cytoplasm had become more granular and in some areas was hydropic or pale-staining. Fat droplets (Fig. 2) appeared in cells near the center of the lobule. Sometimes there were clear halos in the perinuclear cytoplasm. At this stage the sinusoids in the centers of the lobules were already slightly distended with red blood cells. In the center of the lobules there were a few small groups of cells in which the cytoplasm was somewhat homogeneous and pink, and the nuclei were pyknotic or failed to stain, indicating small areas of coagulative necrosis.

The most advanced degree of hepatic injury was encountered in animals about 4 hours after intraperitoneal injection. There was marked necrosis involving the centers of the lobules. The extent of this necrosis varied from a few cells to almost the entire lobule. There was complete dissociation of the liver cords with remnants of liver-cell bodies lying free in the blood of the confluent sinusoids. These isolated liver cells were usually rounded and swollen. The cytoplasm was hyalinized, eosinophilic, and sometimes vacuolated as a result of fat accumulation. The fat droplets were always small, being from 4 to 8 μ in size. The nuclei of these cells were pyknotic or failed to stain. In some instances the liver cords at the center were partly preserved in form and also contained viable appearing cells, but in many of the more severely injured livers there were collections of a pale blue, homogeneous material lying in pools in the centers of the lobules where the liver cells were completely absent (Fig. 3). This blue material frequently was found to be continuous with adjacent liver cells. A few polymorphonu-

clear leukocytes and also pyknotic nuclei were present in this material. The cells remaining at the periphery were swollen, granular, slightly vacuolated with fat, and also contained some vacuoles devoid of fat.

At this relatively early stage there was but little removal of the dead cell bodies. The sinusoids at the center were markedly distended, apparently confluent, and filled with red blood cells (Fig. 4). There was actual destruction of the sinusoidal endothelium in some instances and the sinusoidal spaces had become confluent, leading to large lakes of blood.

At 24 to 48 hours the number of dead liver cells was markedly diminished and the architecture of the lobule fairly well restored. The Kupffer and endothelial cells at the center of the lobule were present and apparently formed continuous sinusoids. The parenchymal cells were still swollen, granular, and contained many fine fat droplets. The amount of blood in the sinusoids was very greatly reduced compared to the earlier stages.

At 3 to 5 days after an almost lethal dose of the extract, the liver lobules were normal in size and arrangement. Rare coagulative, rounded cell bodies remained around the central vein. The liver cords were intact. The sinusoids contained a normal amount of blood. The liver cells were swollen with somewhat granular cytoplasm, and had enlarged, hyperchromatic nuclei. Mitotic figures were commonly seen in the parenchymal cells, indicating regeneration (Fig. 5).

After 28 days the liver was completely restored to normal as far as histological study could disclose.

Heart. As early as 2 hours after administration of the extract there were swelling and increased granularity of the muscle fibers. Scattered, hyalinized and slightly swollen muscle fibers were present in the left ventricle. Underneath the endocardium and in the myocardium were a few small, recent, interstitial hemorrhages and a more diffuse acute hyperemia.

These changes were most marked at 4 hours, diminishing in intensity thereafter, until at 3 to 5 days there were no demonstrable changes.

Lungs. The lungs were usually only slightly involved. The presence of acute hyperemia was variable. In some instances the alveolar capillaries were actually smaller, in others they were definitely distended. The most common finding was focal, slight, recent alveolar hemorrhage. In a few animals both alveolar and interstitial edema were present.

Kidney. The earliest alteration in the histological appearance of the kidneys was noted 20 minutes after injection. This consisted of a marked, patchy, cortical and medullary hyperemia (Fig. 6). Somewhat later there were swelling, increased granularity, and loss of dis-

tinct luminal border of the convoluted-tubular epithelial cells. In some instances there was necrosis involving only small groups of cells and segments of convoluted tubules. Hydropic swelling and apparent cell disintegration of some of these necrotic cells were observed. A constant finding was the presence of a blue, almost homogenous but finely granular, often loose or reticulated deposit within the lumina of the tubules.

In some rats the cytoplasm of degenerated convoluted tubular epithelium contained small brownish granules resembling bile pigment. Occasionally, hyaline droplets were noted in the cytoplasm.

Sections of spleen, suprarenal glands, intestine, and brain revealed no changes.

Discussion

As indicated by these observations, the toxic substance of *M. aeruginosa* is a rapidly acting cytotoxic agent with particular tendency to damage hepatic parenchymal cells. Its action, however, is not confined to the liver, but leads also to cellular degeneration and necrosis in a much less intense form in other organs, particularly the heart and kidneys. The early occurrence of parenchymatous degeneration of these organs before actual necrosis is present in the liver seems to indicate that the muscle fibers of the heart and the epithelium of the kidney are injured directly by the poison rather than as a result of the hepatic necrosis which follows. The development of petechial hemorrhages in the heart and lungs might indicate that this agent also injures capillary endothelial cells, and the effect of this may be exaggerated by the prolonged clotting time of the blood. The same mechanism might conceivably account for the hyperemia of the various organs, but circulatory failure would seem to be a better explanation.

The first stage of hepatic injury can be considered as the pre-necrotic stage in which progressive degenerative changes in the parenchymal cells are in evidence. The extreme rapidity of action of the toxic substance is indicated by advanced parenchymal degeneration as early as 15 minutes after injection. This rapid response suggests that the poison acts directly upon liver cells without any intermediate changes in the body. The first structural change brought about in the liver cells is an increase in their size, apparently due to increased fluid content. This is accompanied by increased granularity of cytoplasmic substance and, shortly afterwards, by the appearance of fat droplets and fat-free vacuoles within the cytoplasm. The immediate explanation for these alterations is not readily apparent, but it is possible that, as a result of the direct action of the toxin upon the cells, there is increased osmotic pressure within the cells leading to intracellular edema, and that

metabolic processes are altered in such a way that unutilized fat collects in the cytoplasm.

The second stage of injury in the liver is that of necrosis and engorgement. The necrosis begins in the center of the lobule, perhaps because of poorer oxygen supply in this region. The steps leading to necrosis of parenchymal cells can be readily traced from acute parenchymatous to fatty degeneration, and, with or without the intervention of hydropic change, to coagulative necrosis. A remarkable feature is the rapid liquefaction of dead cells. Within 4 hours there is advanced disruption of the cell cords, indicating that many dead cells have been removed. Very striking is the stage intermediate between coagulative necrosis and cytolysis, in which masses of blue-staining cytoplasm lie free in the sinusoids, the cell membranes having apparently disintegrated. This liberation of cytoplasm into the sinusoidal spaces probably leads to a rapid removal of liver parenchymal substance by solution in, or disintegration into, the blood within the lobule. The possible consequences of this liberation of dead liver-cell cytoplasm into the blood stream have not yet been assessed. Associated with necrosis is a marked accumulation of blood within the center of the lobule. This collection of blood is considered to be located largely within markedly widened or hyperemic sinusoids, but in some instances sinusoidal walls have apparently disintegrated with hemorrhage occurring within the lobule. Collection of blood in the centers of lobules is not entirely dependent upon widening of sinusoids from disappearance and thinning of liver cords, for the lobule as a whole is increased in size. The blood which collects in this manner may be of sufficient volume to be an important factor in the circulatory failure which occurs terminally in the acutely poisoned animals.

The next stage of hepatic injury is that in which there is effective removal of dead cells. This appears to proceed rapidly, having begun as indicated about 4 hours after administration of the extract. About 24 hours following administration the lobule is collapsed and smaller. Most of the dead cell bodies have apparently been removed by autolysis or by the cytoplasm being carried away in the sinusoidal blood. At this time, however, there is still some persistence of the parenchymal damage as shown by acute parenchymatous and fatty degeneration in the liver cells which remain. Following removal of dead liver cells, the period of regeneration becomes evident at 3 to 5 days. Regeneration apparently proceeds within the lobule, the general architecture of which remains intact. Regeneration is indicated by restoration of the cell cords, by increased numbers of sinusoidal endothelial cells, by hypertrophy of parenchymal cells, and by mitotic figures. The formation of

new parenchymal cells takes place from pre-existing liver cells. Bile duct regeneration plays no part in this process, nor does it appear to occur here in any form. The process of regeneration and restoration proceeds so that at 28 to 30 days there is no evidence of the previous hepatic injury. The lobules are completely reconstituted, being normal in size and architecture. There is no evidence of nodular regeneration or fibrosis.

This completeness of restoration would seem to be of significance in connection with the problem of hepatic cirrhosis developing from severe injury. With injury of the type produced in these experiments, which is intense with widespread necrosis, it appears that a single episode without persistence of the damaging agent does not lead to cirrhosis, but, instead, to complete restoration of the liver substance. This observation is in accord with that of Lucké [5] who found complete return to normal structure in the livers of patients who had had acute epidemic hepatitis with necrosis of the liver, and also agrees with many studies regarding acute hepatic injury produced by such agents as chloroform and carbon tetrachloride.

The matter of renal injury deserves special comment. The degenerative changes occurring in the convoluted tubules suggest the excretion of substances capable of damaging the tubular epithelium. Bile pigment and bile salts are almost certainly eliminated in increased amounts in the urine of these rats, and in a few the renal epithelium contained granules of bile pigment. However, bile-stained casts and abundant bile pigment in the epithelium were usually missing, and therefore probably did not account for the tubular changes. A second possible cause is the excretion of cytoplasmic products from liquefied liver cells. The blue-staining granular deposit in the tubules suggested such material, but it was not positively identified. Another possible cause of the renal injury is the toxic substance itself, as previously suggested. The fate of the poison within the body, with possible elucidation of the renal injury, is to be studied.

The pathological findings afford some suggestions as to the mechanism of death. There seem to be four separate groups of changes, which individually or collectively might be concerned. First, the necrosis of the liver is so extensive that acute hepatic insufficiency might cause death. Rats dying 24 hours or more after the injection exhibit signs suggestive of hepatic coma. Moderate to profound lowering of the glucose of the blood has been observed at this stage. However, it is improbable that deaths occurring only a few hours after the injection are due to liver insufficiency in this sense. The development of a state of shock in animals dying early, with decreased blood pres-

sure and hypothermia, may account for death. The mechanism of the shock associated with this poisoning has not been completely elucidated, but two probable factors are the collection of blood in the liver and the associated vasodilatation and/or hyperemia of other organs. Another condition which might account for some delayed deaths is that of renal insufficiency consequent to acute tubular degeneration. Finally there is possibly a depression of cellular metabolism in all organs of the body as indicated by parenchymal degeneration, although no such effect could be demonstrated in an earlier study in terms of oxygen consumption of isolated tissues to which extract containing the toxic substance was added, according to the Barcroft-Warburg technic.[4] These observations on the mechanism of death are provisional, being dependent upon deductions from structural changes.

The hepatic injury produced by the poison of *M. aeruginosa* is distinguished from that brought about by a variety of hepatotoxic agents chiefly by the remarkable rapidity of the development of the lesions, the quick disintegration of dead liver cells, and the intense engorgement of the liver with blood. Necrosis, chiefly of the center of the lobules, and the associated parenchymal degeneration and regeneration of the lobules in recovery are observed following administration of chloroform,[6] phosphorus,[7] and numerous other substances [7-9] and in epidemic hepatitis. A considerable similarity exists between this algal poison and the thermostable amanita toxin of the mushroom, *Amanita phalloides*,[10-12] in respect to pathological effects, chemical properties, and composition.[13] Marked increase of tolerance to algal extract after repeated administration is observed just as with amanita-toxin, and other evidence indicates that the active substances may be similar, if not identical.[13]

Various observations in connection with chronic administration of algal poison will be reported subsequently.

Summary

1. The toxic substance which can be extracted from *Microcystis aeruginosa* leads, upon administration to rats, to generalized cellular damage with particularly severe injury to the parenchymal cells of the liver.

2. The injury in the liver can be followed through successive stages of acute parenchymatous, hydropic, and fatty degeneration, to necrosis in the centers of the lobule. The dead liver cells disintegrate rapidly by cytolysis and by fragmentation of cytoplasm into the circulating blood. There is marked engorgement of the centers of the lobules.

3. The dead liver cells are removed at 24 to 48 hours, regeneration is in progress at 3 to 5 days, and there is complete restoration of liver lobules within 30 days after administration of the extract.

4. There is no evidence of nodular regeneration or fibrosis from a single severe injury produced by this hepatotoxic agent.

5. Acute parenchymatous and hydropic degeneration, and focal necrosis as well as hyperemia are observed in the heart and kidneys. Small hemorrhages and occasionally edema occur in the lungs.

6. Early death from acute poisoning can probably be attributed to shock and circulatory collapse. Delayed death is probably consequent to hepatic insufficiency, renal failure, and generalized cellular damage by the toxic agent.

7. The types of structural alterations in the liver produced by the agent are similar in many respects to those produced by several known hepatotoxic substances.

The photomicrographs were prepared by Mr. Lewis Waters of the Medical Arts Department of the Southwestern Medical School.

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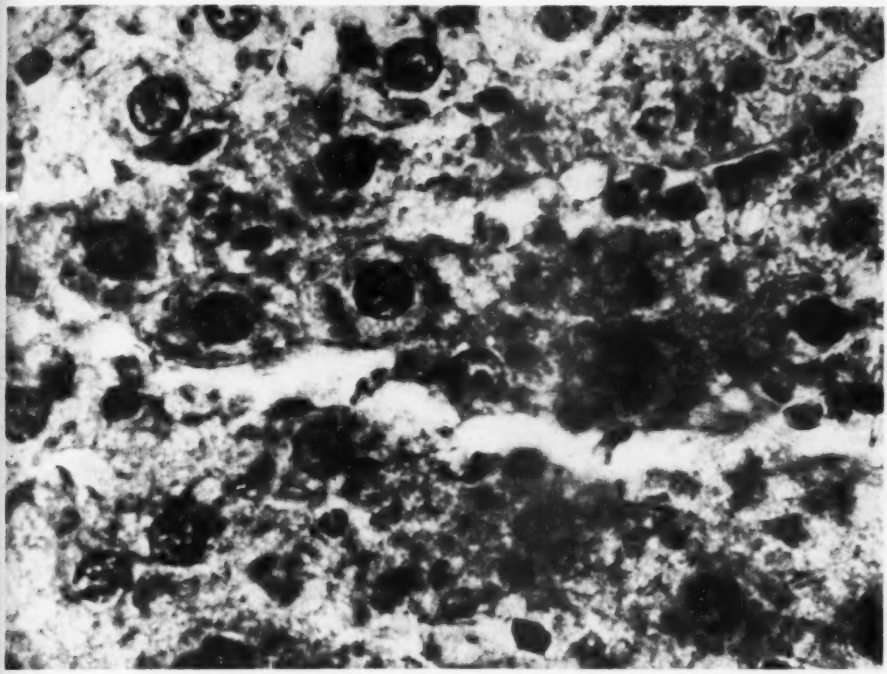
DESCRIPTION OF PLATES

PLATE 83

FIG. 1. Early parenchymatous degeneration of liver, 15 minutes after injection of algal extract. Hematoxylin and eosin stain. × 1170.

FIG. 2. Fatty degeneration at center of lobule, 30 minutes after injection. Hematoxylin and eosin stain. × 380.



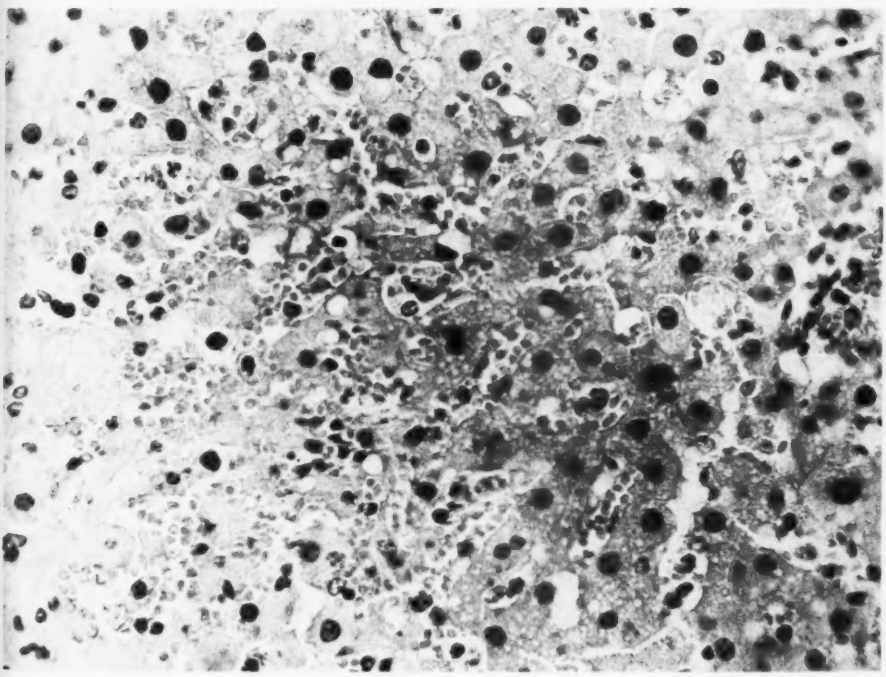
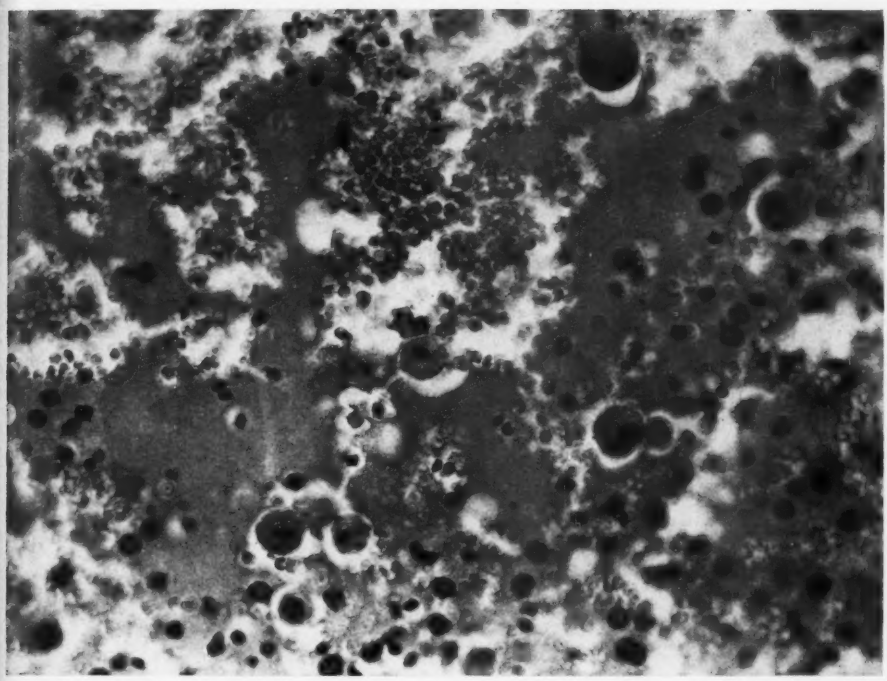


PLATE 84

FIG. 3. Early stage of central necrosis, with cytoplasmic material lying free in sinusoids. Hematoxylin and eosin stain. × 380.

FIG. 4. Marked accumulation of red blood cells in sinusoids in necrotic portion of liver lobules. Hematoxylin and eosin stain. × 380.



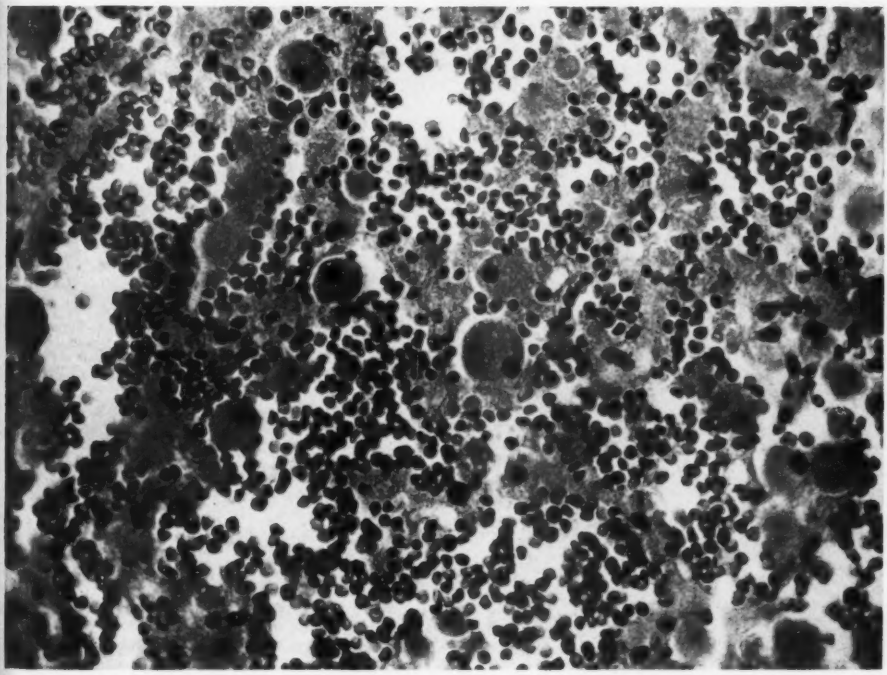


PLATE 85

FIG. 5. Regeneration of liver cells, 5 days after sublethal injection of algal extract. Hematoxylin and eosin stain. × 585.

FIG. 6. Acute passive hyperemia and parenchymatous degeneration in kidney 5 hours after administration of the extract. Hematoxylin and eosin stain. × 380.

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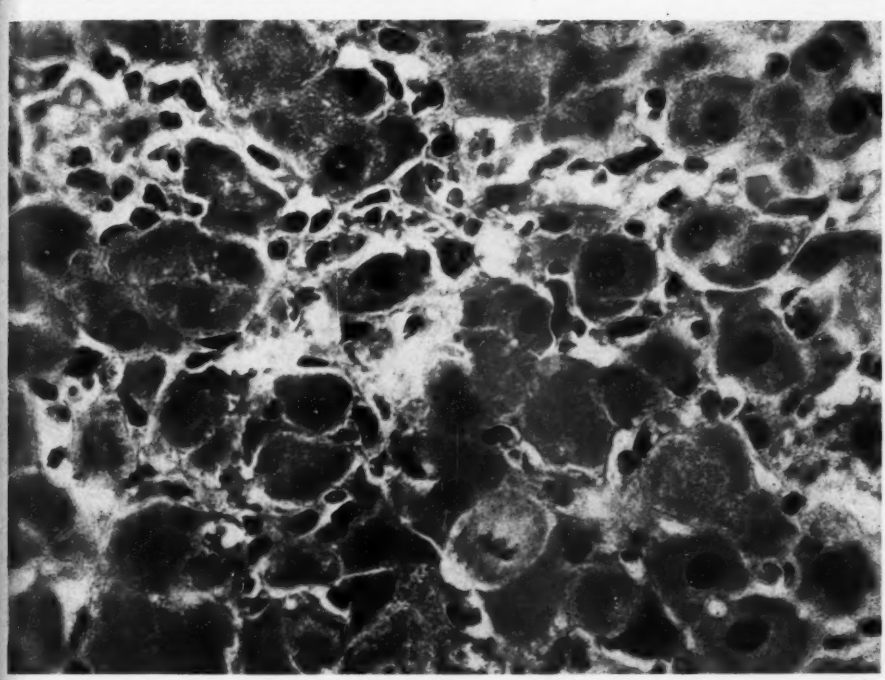

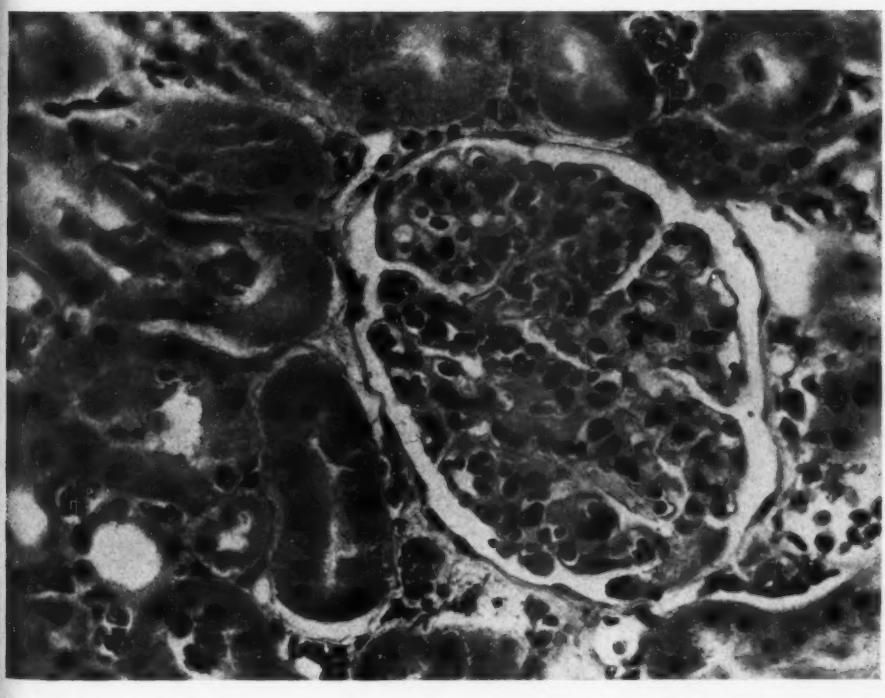

THE NEPHROTOXIC ACTION OF dl-SERINE AS RELATED TO CERTAIN DIETARY FACTORS *

ROBERT P. MOREHEAD, M.D., WILLIAM H. FISHMAN, Ph.D., and CAMILLO ARTOM, M.D.

(From the Division of Pathology of the Department of Pathology and Bacteriology, and from the Department of Biochemistry, The Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N.C.)

Renal necrosis in rats receiving *dl*-serine by stomach tube has been described in a previous report.[1] In those experiments one group of animals was maintained on a stock diet which was considered adequate, while another received a synthetic diet known to be deficient in the B vitamins and low in protein. Within a few days after the administration of *dl*-serine was begun, both groups of rats developed renal necrosis. Although the amino acid was still supplied daily, the animals maintained on the stock diet showed rapid and almost complete repair of the kidney tubules, while those on the deficient diet developed progressive necrotic lesions which were associated with calcification. In the latter group regenerative processes were also apparent, but the injury was so extensive that restoration of the renal parenchyma to its previous state was impossible.

Among the other findings noted was increased capillary permeability, progressing in certain instances to hemorrhagic transudation; these vascular changes were much more pronounced in the animals maintained on the deficient diet. Since the fatalities which occurred were confined to this group, peripheral circulatory failure was suggested as a possible mechanism of death.

The pathological findings after serine administration confirmed the results of previous experiments in which the clinical symptoms and the mortality were studied.[2,3] In an effort to explain the less injurious action of serine upon rats on the stock diet, possible deficiencies of the experimental diet were taken into consideration. It was felt that the diet was deficient in B vitamins, choline, cystine, and glycine.

A later study showed that supplementing the experimental diet with a mixture of pure B vitamins alleviated the severity of clinical symptoms and considerably reduced the mortality in rats receiving serine by stomach tube.[4] Of the individual B vitamins tested, pyridoxine seemed to be most effective. The best results, however, were obtained in rats maintained on the experimental diet supplemented with choline, cystine, glycine, and the complete mixture of B vitamins.

In the present study similar experiments have been performed with

* Aided in part by a grant from the John and Mary R. Markle Foundation.
Received for publication, April 11, 1945.

the object of determining whether the pathological changes induced by serine administration are also influenced favorably by these various dietary factors.

Material and Methods

Male albino rats of the Rockland strain, weighing approximately 100 gm. each, were divided into four groups and placed on an experimental diet (diet 4), the composition of which has been indicated previously.[1] The animals in group 1 received a supplement of 50 μg. of pyridoxine daily. For the rats in group 2 the supplement consisted of a mixture of B vitamins, containing 50 μg. of thiamine hydrochloride, 50 μg. of riboflavin, 1,500 μg. of nicotinic acid, 200 μg. of calcium pantothenate, 200 μg. of inositol, and 200 μg. of p-aminobenzoic acid daily. The animals in group 3, in addition to these B vitamins, received also 50 μg. of pyridoxine daily. Rats in group 4 received the same complete B vitamin mixture as those in group 3, and, in addition, were given daily supplements of choline hydrochloride, *l*-cystine, and glycine (50 mg. each). The vitamins were administered parenterally, whereas choline, cystine, and glycine were incorporated into the diet. As far as possible, the food was supplied to the animals in amounts slightly in excess of their requirements. Food consumption was checked daily, and the rats were weighed every third day. Water was given *ad libitum*.

After the animals of all groups had been maintained on the experimental diets for a period of 7 days, they received by stomach tube 100 mg. of *dl*-serine, dissolved in 3 cc. of water, given daily for a maximum of 14 days. A small number of rats in each group were designated as controls and did not receive the amino acid.

Surviving animals of all groups were sacrificed by decapitation at various intervals (Tables I to IV). Representative tissues from each rat were placed in a 4 per cent solution of neutral formaldehyde. The pieces of tissue were embedded in paraffin, cut at 6 μ, and stained with hematoxylin and eosin.

Results

Examination of the accompanying tables reveals that none of the dietary substances tested were capable of preventing the renal and vascular changes which ordinarily follow the administration of *dl*-serine to animals on the experimental diet (diet 4). The renal lesions were those of prompt necrosis and extensive calcification in the tubules (Figs. 1 to 4), associated with proliferation of fibroblasts, tubular dilatation with regeneration of epithelium, and mononuclear, eosinophilic, and neutrophilic cellular infiltration. In 5 of the 11 animals employed as controls, the kidneys showed calcium deposits in the form

of flecks and sheets, but these deposits in most instances were minimal in degree (Fig. 5).*

The vascular changes following serine administration consisted of general capillary dilatation, presumably associated with increased permeability. Red blood cells were present in the pulmonary alveoli of most of the animals and varied in amount from minute hemorrhagic foci scattered throughout the lung substance to almost complete hemorrhagic consolidation (Fig. 6). None of the substances tested appeared

TABLE I

Experimental Diet + Pyridoxine + Serine

No. of animal	Duration of experiment	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
	(*days*)					
A-34-3	9	1	Calcification	+	—	+
A-34-4	9	1	Necrosis, calcification	++	+	—
A-33-5	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	+
A-33-6	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	+
A-33-1	22	14	Tubular dilatation and regeneration, calcification	++	—	—
A-33-2	22	14	Tubular dilatation and regeneration, calcification	—	—	—
A-33-3	29	14	Tubular dilatation and regeneration, calcification	++++	—	+
A-33-4	29	14	Normal kidneys	+++	+	—
Controls						
A-34-5	8	0	Calcification	—	+	—
A-34-6	8	0	—	—	+	—
A-31-5	21	0	—	—	+	+
A-34-1	28	0	—	—	+	+
A-34-2	28	0	—	—	—	+

to affect the process. In the control animals, however, vascular changes were either absent or minimal in degree.

Myocarditis was present in approximately 50 per cent of the animals, and occurred in all groups. The lowest incidence, however, was found in the group of animals receiving the complete B-vitamin supplement (group 3). The rats of group 4, on the other hand, showed a high incidence of myocardial disease, although they had received supplements of choline hydrochloride, cystine, and glycine in addition to the complete mixture of B vitamins. The disease was characterized by a degeneration of the myocardial fibers and mononuclear infiltration (Fig.

* The changes in the kidneys of the animals employed in this study were identical with those described in a previous report[1] and therefore have not been illustrated in detail here.

7). Eosinophils and neutrophils were seen in small numbers, and there was some fibroblastic activity. The process was most marked in the auricles.

TABLE II

Experimental Diet + Complete B-Vitamins Mixture — Pyridoxine + Serine

No. of animal	Duration of experiment	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
	(*days*)					
A-31-1	9	1	Necrosis	—	+	—
A-31-2	9	1	Necrosis	++	—	+
A-32-5	13	5	Necrosis, tubular dilatation and regeneration, calcification	+	—	—
A-32-6	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	+
A-32-1	22	14	Tubular dilatation and regeneration, calcification	+++	+	+
A-32-2	22	14	Tubular dilatation and regeneration, calcification	++	+	+
A-32-3	29	14	Calcification	Multiple abscesses	+	+
A-32-4	29	14	Normal kidneys	—	—	+
Controls						
A-31-3	8	0	—	—	+	+
A-31-4	8	0	—	++	+	—

With the exception of those rats whose diets were supplemented with choline (group 4), the majority of the animals which received serine developed fatty changes in the liver. These changes consisted in a gradual increase of hepatic fat which later was associated with degenerative changes in the liver.

TABLE III

Experimental Diet + Complete B-Vitamins Mixture + Serine

No. of animal	Duration of experiment	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
	(*days*)					
A-36-1	9	1	Necrosis, calcification	—	—	—
A-36-2	9	1	Necrosis, calcification	++	—	—
A-39-1	13	5	Necrosis, tubular dilatation and regeneration	+++	—	+
A-39-2	13	5	Necrosis, tubular dilatation and regeneration, calcification	—	—	—
A-37-4	22	14	Tubular dilatation and regeneration, calcification	++	—	+
A-37-6	22	14	Tubular dilatation and regeneration, calcification	—	+	+
Controls						
A-36-3	8	0	Calcification	++	—	+
A-36-4	8	0	Calcification	—	—	—
A-37-1	21	0	Calcification	—	—	+
A-37-2	21	0	Calcification	—	+	+

The findings with respect to the clinical appearance of the rats, changes in weight, and food consumption in the present experiments closely resembled those previously described.[4] Since animals were sacrificed at intervals, no statement can be made regarding the mortality in the various groups.

Discussion

It was clear from our previous experiments [1, 2] that both clinical symptoms and pathological alterations induced by serine were less marked in animals on the stock diet than in those on the experimental diet. Among the possible factors which may favor the nephrotoxic action of *dl*-serine, the low protein level of the experimental diet (diet 4) was considered. In earlier experiments, however, the casein content of

Table IV

Experimental Diet + Complete B-Vitamins Mixture + Choline + Cystine + Glycine + Serine

No. of animal	Duration of experiment	Days receiving serine	Degenerative kidney disease	Hemorrhagic transudation (lungs)	Myocarditis	Fatty liver
	(*days*)					
A-41-1	8	1	Necrosis	+++	—	—
A-41-5	8	1	Necrosis	++++	+	—
A-40-5	13	5	Normal kidneys	++	—	—
A-40-6	13	5	Necrosis, tubular dilatation and regeneration, calcification	++	+	—
A-41-3	14	6	Tubular dilatation and regeneration, calcification	+++	+	—
A-40-1	15	7	Tubular dilatation and regeneration, calcification	—	—	—

the experimental diet had been increased from 10 per cent (diet 4) to 30 per cent (diet 2).[2] Prior to the administration of serine, this diet provided a very satisfactory rate of growth, almost identical with that of animals on a stock diet considered adequate. However, when serine was administered, the additional amount of casein apparently made no difference in the severity of the clinical symptoms or in the mortality. The autopsy findings in the animals on this diet which succumbed during the experiments (unpublished data) did not differ qualitatively or quantitatively from those in animals which had received only 10 per cent casein in their diets. It was therefore suggested that the protective action of the stock diet against the toxic effect of serine was not due merely to the maintenance of a state of good nutrition but rather to some specific factor(s) in the diet. The experimental diet (diet 4) was almost certainly deficient in choline, cystine, glycine, and B vitamins. It appeared reasonable to determine whether or not one or more of

these substances could modify the severity of the pathological changes induced by serine.

In the experiments which constitute the basis of this report, white male rats weighing approximately 100 gm. were divided into four groups and placed on diet 4 for the duration of the experiment. The vitamins and other substances mentioned were administered as outlined in the tables. During the course of the administration of serine, animals in all four groups developed pronounced changes in their kidneys similar to those which have been described in animals maintained on an experimental diet deficient in the substances mentioned above. In none of the groups did the supplementary substance or substances administered appear to alter in any way the nephrotoxic action of the amino acid.

This finding is in contrast to the definite beneficial effect of the B vitamins, especially pyridoxine, as judged by changes in body weight, food consumption, clinical appearance, and mortality.[4] Apparently this beneficial action is exerted by means of an extra-renal mechanism.

On the other hand, since the renal damage caused by serine is actually less severe in the animals on the stock diet, one may postulate the existence of some factor in this diet (other than those tested) directly protecting the kidney.

Areas of calcium deposit were present in the kidneys of certain of the control animals. This was to be expected, however; for it has been pointed out that rats on the experimental diet alone (diet 4) develop small calcium deposits in their kidneys.[1] Renal calcification has been noted under a variety of experimental conditions,[5] and Hummel and Barnes [6] have described calcium deposits in the kidneys of rats maintained on a basal diet adequate in all respects except for caloric supply.

In the present studies no significant differences in the vascular changes were observed among the various groups of animals. It must be remembered, however, that the material consisted only of surviving animals. If the mechanism of death following the administration of serine is peripheral circulatory failure, then the most severe cases would not have been included. On this basis the mortality figures should reflect the severity of the vascular lesions. Accordingly, the hypothesis cannot be excluded that this may be the extra-renal mechanism through which the B vitamins may exert their protective action.

Myocarditis was present in all groups and therefore cannot be considered the result of serine administration. In other studies [1] it was noted among all groups of animals, including those on a stock diet which did not receive serine and which were sacrificed as controls. The higher incidence of myocarditis among the rats of groups 1 and 2 may

suggest, however, a relationship between the diet and the incidence of myocarditis in rats. In this connection, Ashburn and Lowry [7] have described a high incidence of myocarditis in thiamin-deficient rats.

Summary and Conclusions

The administration of ample amounts of B vitamins to rats maintained on an experimental diet did not alter quantitatively or qualitatively the toxic action of *dl*-serine on renal tissue. Identical findings were obtained in rats on the experimental diet supplemented with choline hydrochloride, cystine, and glycine, in addition to the complete B-vitamins mixture. Increased capillary permeability followed the administration of *dl*-serine in all groups of rats. These findings are discussed in connection with those of previous experiments in which these substances were found to alleviate the clinical symptoms of serine injury.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 86

FIG. 1. A section of the kidney removed from an animal belonging to group 1. This animal was sacrificed after having received *dl*-serine on 5 successive days. A destructive lesion is seen at the junction of the cortex and medulla, as well as extensive calcium deposits and dilated tubules. × 8.

FIG. 2. Similar lesion from a kidney of an animal belonging to group 2. × 8.

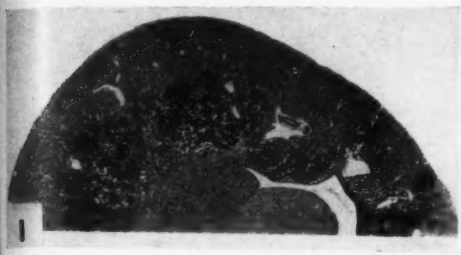
FIG. 3. Similar lesion from a kidney of an animal belonging to group 3. × 8.

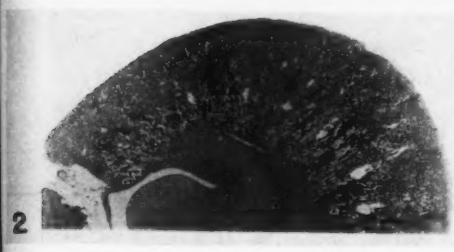
FIG. 4. Similar lesion from a kidney of an animal belonging to group 4. × 8.

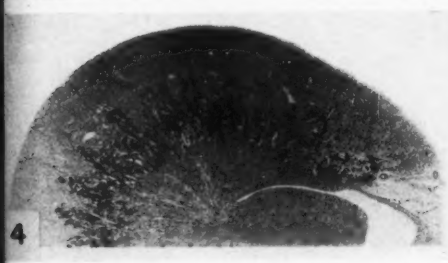
FIG. 5. Section of kidney removed from an animal employed as a control for group 3 and sacrificed after having been on the experimental diet for 21 days. No morphological changes can be seen in this section. × 8.

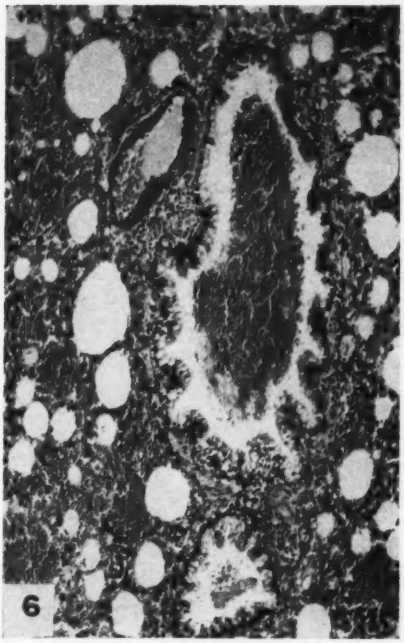
FIG. 6. Hemorrhagic pulmonary transudation. The bronchioles as well as some of the alveoli are filled with blood. × 110.

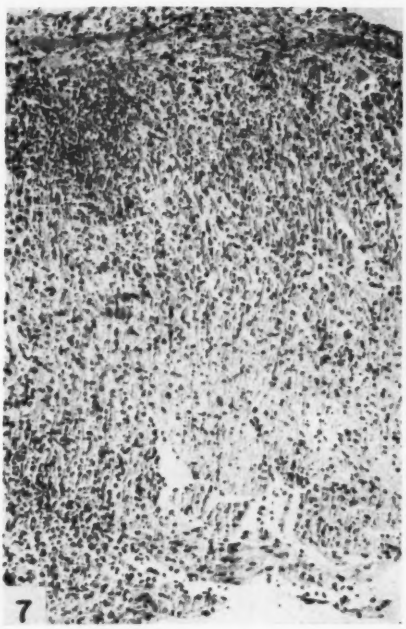
FIG. 7. Myocarditis. There is mononuclear infiltration and myocardial degeneration of the auricle. × 110.











Morehead, Fishman, and Artom Nephrotoxic Action of dl-Serine

"CEROID" PIGMENT IN HUMAN TISSUES *

ALWIN M. PAPPENHEIMER, M.D.,† and JOSEPH VICTOR, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and from the First Division, Research Service, Goldwater Memorial Hospital, New York, N. Y.)

The term "ceroid" was first applied by Lillie, Daft, and Sebrell [1] to a coarsely globular, yellow, wax-like pigment found in the cirrhotic livers of rats maintained on a low protein, low fat diet. The pigment is characterized by its insolubility in lipoid solvents, its affinity for fat stains and for basic aniline dyes, methyl green in particular, its acid-fastness when stained with the Ziehl-Neelsen method, even after prolonged exposure to acid-alcohol, and its fluorescence. Further studies of its properties and association with dietary cirrhosis have been made by György and Goldblatt,[2] Blumberg and McCollum,[3] Blumberg and Grady,[4] Edwards and White,[5] György,[6] Popper, György, and Goldblatt,[7] and Endicott and Lillie.[8]

Female rats on a vitamin E-deficient diet develop brownish discoloration of the uterus. The pigment is deposited in the smooth muscle cells, and within large phagocytes in the intermuscular connective tissue (Fig. 1). This very striking and characteristic pigmentation has been studied by Martin and Moore,[9,10] Barrie,[11] Hessler,[12] Demole,[13] Barrie-Sweeten,[14] and by Mason and Emmel.[15] In a recent paper, Mason and Emmel[16] found similar pigment in ovaries and in the interstitial tissue of the testis. They called attention to the resemblance of the pigment to "ceroid" and stated that further studies on its identification were in progress. Moore and Wang [17] and Mason and Emmel‡ have shown that, like "ceroid," the uterine pigment is fluorescent. It has been found in our laboratory and in that of Dr. Mason that the uterine, ovarian, and testicular pigment in vitamin E-deficient rats is acid-fast, and stains selectively with methyl green, thus indicating its "ceroid" character. Similar pigment is present in the motor ganglion cells of the cord and medulla of older rats (Figs. 2 and 3), maintained for long periods on a vitamin E-deficient diet, and is regularly absent in controls receiving wheat germ oil or tocopherol (Table I). We have also noted pigment with identical staining reactions in the atrophic testis of a vitamin E-deficient guinea-pig [18] (Fig. 4). It was located within degenerating germ cells, giant cells, and Sertoli cells of the tubules, but not in the Leydig cells or intertubular stroma.

* Received for publication, April 19, 1945.
† Now at 5 Acacia St., Cambridge, Mass.
‡ Personal communication.

In 1917, Whipple and Hooper [19,20] called attention to pigmentation of the intestinal muscularis in dogs with biliary fistulae, and later they noted a similar pigmentation in dogs which had been rendered anemic by bleeding. Nachtnebel,[21] in 1933, re-investigated this pigment, noted a similarity to hemofuscin, and, since the dogs had been fed liver and cod-liver oil, was inclined to attribute it to the absorption of some dietary constituent from this source.

From the microchemical reactions carried out by Nachtnebel,[21] it is impossible to identify the pigment as "ceroid," although the staining with basic fuchsin and Nile blue sulphate is consistent with "ceroid." Through the courtesy of Dr. Karl Mason, we have had opportunity to study a section of dog's intestine from Dr. George H. Whipple's material. The muscle cells were loaded with fuchsinophile, acid-fast gran-

TABLE I

"Ceroid" Pigment in Ganglion Cells of Vitamin E-Deficient Rats

No. of rats	Diet	Age	"Ceroid" pigment			Testicular atrophy
			Brain	Medulla	Spinal cord	
		(months)				
8	—E	7–12½	—	+(8)	+(8)	+
8*	+E	18–20	—	—	—	—

* Of the controls, 2 received a stock diet; 1, 3 gtt. of wheat germ oil; 1, 6 gtt. of wheat germ oil daily; 4, 5 mg. of synthetic *dl*-alpha-tocopherol weekly.

ules, and these were present also within large phagocytic cells. The appearance was identical with that found in the human cases reported below. Because of its staining with the Ziehl-Neelsen method, this pigment may also be tentatively identified as "ceroid."

It is interesting in this connection that dogs with chronic biliary fistulae have been found by Brinkhous and Warner [22] to develop muscular dystrophy and testicular atrophy, and this is ascribed to failure of absorption of vitamin E when bile is excluded from the intestine. These dogs also, Dr. Mason informs us, contain an abundance of "ceroid" in their intestinal musculature.

So far as we are aware, the occurrence of "ceroid" pigment in human tissues has not been recorded. György [6] made the flat statement that "ceroid" in cirrhotic livers distinguishes the experimental cirrhosis produced in rats by purely dietary means from all other forms of cirrhosis in animals and man. Lillie and his collaborators [23] were unable to find "ceroid" in experimental selenium cirrhosis or in human cirrhosis. Similar negative results were obtained in investigations carried out by

Popper, György, and Goldblatt.[7] More than 200 normal and pathological human livers were examined for "ceroid," including many livers with cirrhosis, acute diffuse necrosis, and hemochromatosis. The only exception to this statement is that in lipoid pneumonia caused by cod-liver oil, the lipoid droplets in the alveolar exudate are known to retain fuchsin after acid decolorization (Pinkerton,[24] Graef [25]). It has been shown recently by Endicott [26] that prolonged oxidation *in vitro* of cod-liver oil or linseed oil with potassium bichromate renders them acid-fast; and Haas [27] and Endicott [26] have found that cod-liver oil injected subcutaneously into rats gradually acquires acid-fastness.

Since the presence of acid-fast "ceroid" pigment in human tissues, other than in the cod-liver oil pneumonias, has not been recognized, it may be of interest to present in detail four cases of profound nutritional disorder in which the tissues, and particularly the intestinal musculature, contained a great abundance of this pigment. We shall refer also to the occurrence and distribution of acid-fast pigment in various tissues from routine autopsy cases. Although no studies of the fluorescence of this pigment have been carried out by us, the assumption that the acid-fast pigment seen in paraffin-embedded material is identical with "ceroid" seems justifiable, and we have used the terms interchangeably. All sections were stained with carbol-fuchsin overnight at room temperature, or at 56° C. for 1½ hours, decolorized with 3 per cent HCl in 80 per cent alcohol for 1 to 3 hours, and counterstained with hematoxylin or methylene blue. In some cases, the pigment was shown to be sudanophilic, and to stain intensely with methyl green, a reaction considered by Popper, György, and Goldblatt [7] to be specific for "ceroid." While the significance of this pigmentation cannot be precisely defined in the light of present knowledge, there is, as will be pointed out later, a certain amount of circumstantial evidence indicating a relationship to deficiency of vitamin E.

Case 1

M. M., a female, single American school teacher, was admitted to the Presbyterian Hospital (history no. 511705, autopsy no. 14033) four times between February, 1937, and January, 1943.

Chief Complaint. The patient's chief complaint was swelling of the legs for the past 5 years. One sister had a similar condition. Three siblings were healthy.

Present Illness. About 5 years before admission she began to have swelling of the legs and, about 1 year later, attacks of vomiting before breakfast. There had been occasional episodes of fever and malaise. Later she had edema of the face and scalp, and moderate dyspnea on exertion.

Physical Examination. On examination the temperature was 98.2° F.; pulse, 80; respiration, 20. There was edema of the trunk and extremities. Teeth were widely separated and incisors pegged. Skin was inelastic and doughy, with many striae albicantes. Trousseau and Chvostek signs were positive.

Laboratory Findings. The venous pressure was 67 mm. of water. Chemical examination of the blood gave the following results: CO_2-combining power of the serum, 55.6 vols. per cent; chlorides, 114 mg. per L.; inorganic phosphates, 3.5 mg. per cent; serum protein, 3 per cent; albumin, 1.7 per cent; globulin, 1.3 per cent; calcium, 3.8 mg. per cent; cholesterol, 111 mg. per cent. Hemoglobin was 71 per cent; red blood cells, 3,700,000 per cmm.; leukocytes, 3,160 per cmm. Gastric expression contained no free HCl, 6 per cent combined acids. There was no change after histamine. Phenolsulfonphthalein excretion was 43 per cent after 2 hours. No albumin was found in the urine on numerous examinations. The stools were bulky and fatty at times, but steatorrhea was not a striking feature.

Course. During the patient's stay in the hospital, extensive laboratory studies were carried out. Hypoproteinemia and hypocalcemia persisted, but the edema was somewhat relieved, as were the symptoms of active tetany. After her discharge, she was followed in the out-patient department and then referred to the hospital of the Rockefeller Institute for further studies. The underlying mechanism of her disease was not discovered, and she was referred back to the Presbyterian Hospital. Hypoproteinemia and hypocalcemia persisted, the edema increased, and ascites and pleural effusions developed.

Post-Mortem Examination

The autopsy was performed by Dr. C. Schubert. Only the significant findings need be cited. There was marked subcutaneous edema and little fat. The peritoneal cavity contained 4 liters of pale gray, milky fluid. There were 2800 cc. of similar fluid in the right pleural cavity, 250 cc. in the left. The *lungs* were compressed by fluid, but otherwise normal. The *liver* weighed 1525 gm. and was slightly nodular, particularly in the region of the hilus. The surface was pale, reddish brown, wet and translucent. Connective tissue was slightly increased in the nodular areas. The *spleen* weighed 325 gm., was brick-red and rather soft, and the follicles were indistinct. The *gallbladder* contained a single stone. The aggregate weight of the *parathyroid glands* was 229 mg. The rugae of the *stomach* were well developed and the mucosa was not atrophic. The submucosa of the *small intestine* was edematous, and the muscular coat had throughout a rusty brown color (Fig. 5). The *colon* also was edematous. There were no ulcers and the mucosa grossly was not abnormal. The *heart, esophagus, pancreas, thyroid, kidneys, uterus, tubes,* and *ovaries* showed no abnormality.

Microscopic Examination

Aside from the edema and the abnormal pigmentation to be described below, no lesions of note were found in any of the tissues.

Pigment was abundantly present in the smooth muscle cells of the lower esophagus, stomach, and small intestine. Both circular and longitudinal coats contained it, but not the muscularis mucosae. Large mononuclear cells stuffed with pigment were seen in the perivascular connective tissue between the muscle bundles and in the subserosa.

The pigment granules, which were coarse, stained yellow brown with sudan III, dark purplish blue with methyl green after differentiation with dilute acetic acid (Popper *et al.*[7]) and brilliant red with carbol fuchsin after decolorization with 3 per cent HCl in 80 per cent alcohol (Fig. 6). The individual muscle cells were distended with the granules, appearing much thicker than normal fibers. The muscle of the large intestine did not contain pigment.

In the liver, the "ceroid" pigment was present in the form of coarse droplets within the Kupffer cells. The liver cells contained fine granules of lipochrome pigment which did not give the characteristic reaction with methyl green or Ziehl-Neelsen stains. The uterine muscle contained it sparingly (Fig. 7). In the ovary, there were large accumulations of phagocytes filled with "ceroid," chiefly in the vicinity of stretic follicles (Fig. 8). Pigment granules were found also in individual muscle fibers in the media of small arteries in the thyroid and pancreas. No pigment was seen in the aorta. The muscle cells of the myocardium, however, showed a considerable amount, having the same distribution as the usual lipochrome pigment, but distinguishable because of its acid-fastness. The individual droplets were larger than the slightly more refractile, brownish lipochrome granules which were also present. The skeletal muscle contained sparse numbers of acid-fast droplets at the poles of the nuclei.

No pigment was found in the muscle of the gallbladder, bronchi, or pancreatic ducts. The spleen, lungs, and adrenals were also free.

Comment

The nature of this patient's illness, in spite of intensive clinical studies, was never satisfactorily explained. The hypoproteinemia could not be permanently influenced by diets high in protein, administration of casein hydrolysates, amino acids, or high doses of vitamins. It was never clear whether there was a primary failure of absorption, or inability to utilize amino acids for synthesis. There was no history obtainable of vitamin deficiency.

The literature contains isolated reports of cases of so-called "idiopathic hypoproteinemia."[28-33] However, we have found reference to but one autopsy report on such patients, that of Thompson, McQuarrie, and Bell.[30] This patient, a child, 2 years of age, was found to have atrophic changes in the liver, but there is no reference to the condition of the intestines, nor to the presence of pigment.

The following case also illustrates the extensive deposit of "ceroid" pigment which may occur in association with chronic nutritional disorders.

Case 2

S. B., a taxi driver, 32 years old, was admitted to the hospital (Presbyterian Hospital, history no. 338885, autopsy no. 12281) on four occasions between 1918 and 1936. He first entered because of a duodenal ulcer, diagnosed when he was but 10 years old. A gastro-enterostomy was performed with relief of all symptoms. In 1932, however, he began to have diarrhea and it was shown by x-ray examination that a gastrocolic fistula had developed. From that time until shortly before death, 4 years later, the diarrhea continued, with two to four watery stools daily. He gradually lost strength and weight; there was pitting edema of the ankles and fluid in the abdomen; the cecum and colon became distended. His total serum proteins fell to 4.4 per cent, with the albumin remaining relatively high at 3.3 per cent. There was hypoglycemia. Urine was normal save for an occasional trace of bile. There was no marked anemia.

Post-Mortem Examination

Autopsy was performed on November 16, 1936, 5½ hours after death, by Dr. Holman. The interesting findings were: (1) a persistent small chronic ulcer near the site of the gastro-enterostomy opening; (2) a double opening of the stomach into jejunum and transverse colon, so that much of the ingested food must have passed directly into the large gut; (3) fatty cirrhosis of the liver which gave the impression of having resulted from continuing destruction and replacement of liver cells—many liver cells showed a peculiar coagulative necrosis of their cytoplasm; (4) advanced emaciation, with almost complete disappearance of the subcutaneous fat.

Microscopic Examination

Pigment. Brownish pigment in the form of granules or droplets retaining the Ziehl-Neelsen carbol fuchsin stain after prolonged treatment with 3 per cent HCl alcohol, and staining dark purplish blue with methyl green, was found in the following situations:

(1) The *smooth muscle* of esophagus, stomach, and small intestine. A section taken through the jejunal-colic fistula showed abundant pigment in the jejunal musculature, but none in the muscle of the large gut except in the immediate vicinity of the line of junction. It was not present in the muscularis mucosae. In the stroma of the mucosa, many plasma cells and large mononuclear cells contained clumps of acid-fast material. Pigment was present also in phagocytes in small lymph nodes and intestinal follicles.

(2) *Skeletal Muscle.* A section of the rectus abdominis muscle, which showed necrosis of some fibers, marked atrophy of others, fibrous replacement, and regenerating myoblasts, showed also abundant acid-fast pigment. This was situated within large macrophages or in spindle-shaped, sometimes multinucleate, myoblasts (Fig. 9).

(3) *Testes.* Spermatogenesis was completely lacking, and many of

the tubules contained only irregular syncytial masses of Sertoli cells. In the fibrillar cytoplasm of these cells were scattered globules or clumps of acid-fast pigment. This, however, was much more abundantly present in the cells between the atrophic tubules. Whether the pigment-containing cells were Leydig cells or large macrophages or connective tissue cells was difficult to determine. Although some of the pigment-bearing cells were in close apposition to the basement membrane, passage of the pigment from tubule to interstitium across the membrane was not observed.

(4) *Liver.* There was advanced fatty infiltration of the liver, the majority of the hepatic cells containing a single large globule. With this there was moderate cirrhosis, rather diffuse and irregular, unaccompanied by marked regeneration of liver tissue or proliferation of bile ducts. Large clumps of acid-fast pigment were present both within the liver cells and in Kupffer cells, and in phagocytes in the cirrhotic stroma. The individual globules varied in size, but were considerably larger than the commonly seen fine granules of lipochrome pigment. They stained brilliantly with the Ziehl-Neelsen stain and with methyl green.

(5) *Kidney.* Clumps of coarse acid-fast pigment were located within the epithelial cells of the loops of Henle.

Comment

Since much of the food in this patient passed directly from stomach to large intestine, it is fair to assume that intestinal absorption of proteins, fats, carbohydrates, and fat-soluble vitamins must have been greatly impaired. The type of fatty cirrhosis of the liver with abundant accumulation of "ceroid" pigment closely reproduces the experimental cirrhosis of rats induced by low casein diets. Whether the testicular atrophy and dystrophy of skeletal muscle may be interpreted as evidences of vitamin E-deficiency is an open question, since there is no precise knowledge of the effects of vitamin E lack in human beings. Such a possibility, however, is not to be excluded, and indeed is strengthened by the finding of "ceroid" pigment in tissues where it is deposited in vitamin E-deficient male rats, namely, the testes, smooth and skeletal muscle.

A third case of nutritional disorder, also characterized by pigmentary deposits of this sort, follows.

Case 3

E. C., an American housewife, 42 years old, was admitted to the Presbyterian Hospital (history no 566263, autopsy no. 13884) on five occasions from November, 1938, to May, 1942. Her history, in brief, was as follows:

Chief Complaint. The patient had had diarrhea and weakness since childhood. Since her first pregnancy, 8 years previously, she had suffered from postprandial epigastric pain, soreness of the tongue, and dysphagia. For 3 weeks before admission she had had cramps in the extremities and carpal spasm.

Family History. Irrelevant.

Past History. The patient had always lived in the United States. Her only previous illnesses had been whooping cough in childhood, psoriasis, and sinusitis.

Present Illness. During infancy and childhood, the patient was said to have had dysentery, which was worse during the summer, and to have been anemic. At the age of 28, she felt very weak and was given injections of iron and liver with benefit. During this period, however, she continued to suffer from diarrhea. At the age of 33, she was delivered spontaneously of an 8 months' baby which died within 24 hours. Following this delivery, weakness and diarrhea recurred and, in addition, she complained of a burning sensation in her throat, relieved by vomiting or by lime water. Because of these symptoms and because she wished to have another child, she had consulted Dr. Watson 5 years previously. He found her to have a macrocytic hyperchromic anemia, and also a retroverted, retroflexed uterus. She was admitted and a uterine suspension performed. Upon discharge, she was given liver extract and felt well for 9 months. At that time, she stopped the injections and again developed diarrhea and weakness.

On her next admission in October, 1940, temperature, pulse, and respiration were normal. Her stools were light, semi-liquid and frothy. No ova or parasites were found. She was again given injections of liver extract, with vitamins and one transfusion. Reticulocytes rose to 8.7 per cent and hemoglobin upon discharge was 100 per cent. She remained at home upon a high protein, low fat diet, and was readmitted in April, 1942, for the delivery of a full-term child, which survived. Two weeks later, diarrhea again became severe, she became weak and suffered from numbness, cramps, and twitchings of the extremities. She was again admitted to the hospital.

Physical Examination. Temperature, pulse, respiration, and blood pressure were normal. During examination, she displayed typical symptoms of tetany. She became dehydrated in spite of vigorous intravenous therapy. The blood calcium, which was 6.3 mg. per cent on admission, was raised to 8.7 by administration of calcium gluconate.

The blood protein was never greatly reduced. On her last admission, total proteins ranged between 5.85 and 7.26 per cent, globulins between 1.45 and 2.00, albumin between 4.40 and 5.26. There was thus no significant disturbance of the albumin/globulin ratio. The other laboratory tests threw no further light upon the case. She failed rapidly and died. The diagnosis was *non-tropical sprue.*

Post-Mortem Examination

At autopsy, which was performed by Dr. Beaubien 2 hours after death, there was no edema nor fluid in the serous cavities. All thoracic and abdominal viscera were grossly normal. The intestines were distended with gas; the Peyer's patches were atrophic; there were no ulcers. The colon also was dilated, but not otherwise abnormal.

Microscopic Examination

Pigment, retaining fuchsin after acid decolorization, was found in abundance in the smooth muscle fibers and within large phagocytic cells in the *small intestine.* The *liver* also contained a considerable amount of

pigment, both within hepatic cells and in the Kupffer cells. Some of the granules in the liver cells appeared angular and had a brownish cast; others stained pure red and had the form of droplets. The pigment in the Kupffer cells stained pure red. In the *uterus* there was acid-fast pigment in moderate amount in many of the smooth muscle cells, as well as within large mononuclear phagocytes. These were especially numerous in the stroma of the mucosa. In the *ovary* large collections of "ceroid"-containing phagocytes were seen in the vicinity of vessels and about atretic follicles. The pigment in the reticular zone of the *adrenal* cortex retained the fuchsin after decolorization. There were scattered *skeletal muscle* fibers showing hydropic vacuolar degeneration without reaction. No pigment was found in the muscle.

Comment

This woman suffered from diarrhea with sprue-like stools over many years, associated with macrocytic hyperchromic anemia and hypocalcemia. During her final illness, however, there was no anemia, plasma proteins were within normal limits, and there was no edema.

Bearing on the possibility of a vitamin E deficiency is the death of her first child 24 hours after an 8 months' delivery. The peculiar hydropic degeneration of the skeletal muscle appears to be a terminal change and cannot be regarded as significant.

Case 4

A. U., female, Irish housemaid, single, age 33, was admitted (Research Division, Goldwater Memorial Hospital: history no. 772, autopsy no. A1026) on July 1, 1943, and died on February 6, 1944.

Chief Complaints. The patient complained of swelling of the legs since December, 1941; weight loss, weakness, abdominal cramps, and diarrhea.

Family History. Irrelevant.

Past History. There had been a tonsillectomy for recurrent tonsillitis in October, 1941.

Present Illness. With the onset of edema of the ankles and general weakness in 1941, the patient went to St. Vincent's Hospital, remaining there for 8½ months. Menses ceased 1 month after admission. Painless diarrhea with bulky, watery stools began 2 weeks after her entrance to the hospital and continued until her death. In February, 1943, she was admitted to Bellevue Hospital and in April to the Presbyterian Hospital. At this time, there was no dependent edema. In May, she was sent to the Goldwater Memorial Hospital. She had lost 42 pounds from the onset of her illness to the time of last admission.

Physical Examination. Emaciation, pallor and tenderness in right lower quadrant near iliac crest.

Laboratory Findings. Examination of the blood gave the following data: Red blood cells, 2.7 to 3.0 millions; white blood cells, 7,550 with 10 per cent eosinophils; reticulocytes, 3.5 per cent; serum cholesterol, 191 mg. per cent; serum albumin, 2.1 gm. per cent; serum globulin, 2.3 gm. per cent; bromsulfalein retention, 0 at ½ hour; serum chlorides, 339 mg. per cent; CO_2-combining power, 60 vols. per

cent; blood calcium, 7.8, 7.1, and later, 10.4 mg. per cent; blood phosphates, 3.9 mg. per cent. The urine had a specific gravity of 1.016 to 1.026; albumin was 1 plus; there were no red or white blood cells or casts. Phenolsulfonphthalein excretion was 87 per cent in 2 hours. Guaiac test of the stool was negative; the fat content was 8.6 to 21.0 per cent.

Post-Mortem Examination

Autopsy was done by Dr. J. Victor, 2½ hours after death. The essential gross findings were chronic jejunitis, chronic mesenteric lymphadenitis, emaciation, anemia, osteoporosis, fatty liver, Laënnec's cirrhosis, thrombosis of portal vein (intrahepatic), splenomegaly, anasarca, purpura, hemorrhage in renal pelvis, squamous metaplasia in pancreatic ducts, lobular pneumonia, involution of ovary.

Microscopic Examination

Many signs of malnutrition are included in the diagnoses above. Intracellular granules or droplets retaining carbol-fuchsin stain after prolonged acid decolorization were present at various sites, namely, within histiocytes of mesenteric lymph nodes, germinal follicles of spleen, fibrous scars within the liver, stroma of uterine endometrium, and in the infiltrated and scarred jejunal lesions. Acid-fast granular material was found also in smooth muscle cells of arteries of the spleen and ovary, and in the intestinal muscle of the jejunum near the lesions. It was found also within endothelial cells of capillaries, veins, and arteries in the ovary, and in the myoepithelial cells of the juxtaglomerular bodies of the kidneys.

Comment

This woman suffered from diarrhea, probably due to chronic jejunitis, over a period of 3 years. This resulted in wasting, hypoproteinemia, and undoubted impairment of intestinal absorption. The hepatic changes closely resemble those produced experimentally with low protein diets. There was widespread deposit of "ceroid" pigment in the locations cited.

Distribution of Acid-Fast Pigment

In view of the presence of large amounts of acid-fast pigment in these cases of obvious nutritional deficiency, it seemed imperative to search systematically in different tissues for its occurrence. Pigment having this property of "acid-fastness" has been found in many cases and in various situations, as will become apparent from the following survey.

Heart. The juxtanuclear lipochrome pigment has been found to vary

in its acid-fast staining. In 42 human hearts examined, the pigment was distinctly brown and non-acid-fast in 12 cases, brown with a reddish cast in 16, and distinctly red in 6. It is interesting that the cases in which the pigment had a "ceroid" character were all associated with liver diseases, namely, hemochromatosis with cirrhosis (2) or Laënnec's cirrhosis (4), one of the latter group having, in addition, carcinoma of the stomach. When the pigment was acid-fast, it appeared to occur in larger masses or even as thick rods with rounded ends.

Aorta. Phagocytes filled with brilliantly red-stained, acid-fast droplets have been noted among the foam cells of atherosclerotic plaques and in medial syphilitic scars.

Blood Vessels. Acid-fast pigment droplets have been found in the muscle cells of medium-sized arteries of thyroid, pancreas, spleen, and ovaries. They have also been found within the endothelial cells of venules, capillaries, and lymphatics.

Lymph Glands. Acid-fast pigment may occur within large phagocytic cells.

Spleen. In two cases of hepatic cirrhosis and one of lymphatic leukemia with extensive infiltration of the small intestine, phagocytes containing "ceroid" were found within the malpighian follicles.

Alimentary Tract. Except for the cases reported, no pigment was found in the esophagus, and only twice in small amounts in the muscle cells of the stomach in a case of Laënnec's cirrhosis, and in a child with cystic fibrosis of the pancreas. In addition to the above cases, pigment has been found in the muscle fibers of the small intestine in 4 of 27 cases. The positive cases were of celiac disease (1), Laënnec's cirrhosis (2), and cystic fibrosis of the pancreas (1). In 18 sections of large intestine, no pigment was found. In 1 case, it was present in the lymphoid tissue of the appendix, and in another, in leukemic infiltrations of the ileum.

Liver. The question of the occurrence of "ceroid" in this organ is of particular interest in view of the negative reports of others. In our material, acid-fast pigment could be demonstrated within liver cells, in Kupffer cells, within phagocytes in the scarred areas of cirrhotic livers, and within endothelial cells of small blood or lymph channels (Fig. 10). We have examined 38 cases of Laënnec's cirrhosis, and have found acid-fast pigment in 21, an incidence of 55 per cent. Included in this group are 4 cases of primary liver cell carcinoma. No acid-fast pigment was detected in the neoplastic tissue, although in 3 cases it was abundantly present in the noncancerous tissue. In 6 cases of coarse nodular cirrhosis, 3 showed the pigment. One of the positive cases was clearly due to cinchophen. A case of nontropical sprue had a fatty liver in

which many large phagocytes were filled with coarse acid-fast droplets. Liver sections from a case of hemochromatosis, with a history of recovery from sprue, also contained both "ceroid" and hemosiderin. Two other cases of hemochromatosis did not show pigment in the liver. In 2 of 7 cases of cardiac cirrhosis, pigment was present. Five cases of chronic passive congestion contained none, nor did 11 normal livers.

As in the heart muscle, the lipochrome pigment of liver cells and Kupffer cells often exhibits some degree of acid-fastness. In the cases referred to as positive, only pigment showing brilliant red staining has been included. In 12 miscellaneous cases in which the liver was not diseased, no acid-fast pigment was found. Sections from 6 gallbladders also showed no pigment.

Pancreas. No acid-fast pigment was found in the pancreas in 20 cases examined.

Adrenals. The pigment normally present in the reticular zone of the adrenal cortex is in some cases definitely acid-fast. This was true in 7 of 22 sections examined. The diagnosis in these cases was nontropical sprue (1), leukemia with hemochromatosis (1), and Laënnec's cirrhosis (5). In 5 of the remaining cases, the pigment was brownish red, and in the other 10 there was no retention of carbol-fuchsin after acid decolorization. When present, "ceroid" occurred not only in the adrenal cortical cells, but also within phagocytes in the sinuses.

Kidney. In the kidney the pigment has been seen in two situations: in the juxtaglomerular bodies, in the form of minute granules in the myoepithelial cells; and in the descending loops of Henle. Here the brownish pigment which is so commonly present may in some cases be strongly fuchsinophilic. This appears to be exceptional, and not correlated with the deposit of acid-fast pigment in other tissues.

Urinary Bladder. No pigment has been seen in the muscle of the urinary bladder, even in those cases in which it was abundantly present in the intestinal muscle.

Prostate. No pigment was noted in the prostate of 8 cases.

Testis. The testes from 38 cases were studied. "Ceroid" was conspicuous and abundant in the interstitial cells in 19; in the remaining 19 the pigment showed little or no acid-fastness. It was usually, though not invariably, associated with atrophy of the spermatic epithelium and was contained both in the Leydig cells and in phagocytes near the tunica. An interesting finding was the presence of large droplets of "ceroid" in the interstitium of the testis of a 10-weeks-old infant dying of toxoplasmosis. Droplets of "ceroid" were occasionally seen among the Sertoli cells of atrophic tubules and in the epithelial cells of the rete testis and epididymis.

Seminal Vesicles. The epithelium and subjacent connective tissue cells of the seminal vesicles normally contain an abundance of yellowish brown pigment, the nature of which has not been defined. In 3 of 11 sections it proved to be strongly acid-fast. The pigment was present in both the epithelial cells and in isolated cells in the underlying connective tissue (Fig. 12).

Ovary. Our material includes sections of ovary from 12 cases. Phagocytes laden with acid-fast pigment were found in 6, usually in the vicinity of atretic follicles. In one case there was massive accumulation of the pigment, together with hemosiderin, in the wall of a small cyst.

Uterus. In addition to the case cited above in which the pigment was present in smooth muscle cells of the uterus, pigment was found in endometrial phagocytes in one case of Laënnec's cirrhosis.

Skeletal Muscle. In one case of fatty cirrhosis in an alcoholic patient, "ceroid" was present at the poles of the myolemmal nuclei. The muscle fibers were otherwise normal.

Thyroid Gland. In 18 cases no acid-fast pigment was found in the thyroid gland.

Lung. No acid-fast pigment was found in the lungs.

Pituitary Gland. Much of the brownish pigment normally present in the pars nervosa of the pituitary gland is acid-fast. In a case of hemochromatosis, it was deposited in very large amounts along with granules of hemosiderin (Fig. 11).

Central Nervous System. The occurrence and distribution of acid-fast pigment in the central nervous system has been the subject of a separate communication.[40] It may be stated here that such pigment may be found in abundance in the ganglion cells of cortex, thalamus, medulla, and spinal cord, in microglia and oligodendroglia, and in endothelium and perivascular phagocytes.

Discussion

In trying to interpret the possible significance of this pigment, one is handicapped by the fact that the term "ceroid" has no precise chemical implication. Three substances have been obtained by Reeves and Anderson [34,35] from avian and human tubercle bacilli which are strongly acid-fast. They have been identified as optically active hydroxyacids of high molecular weight. Unlike "ceroid," they are soluble in $CHCl_3$ and ether, so that if similar substances are present in the tissue "ceroids," they are probably in combination with substances which are insoluble in lipoid solvents. It may well be that the property of acid-fastness is not restricted to a single chemically defined substance, and that in the case of the commonly occurring lipochrome or "wear and

tear pigments" one is dealing with a mixture of substances. One may indeed find granules of brown and red pigment intimately commingled in the same cell, and one may observe all transitions in staining between brown and pure red. As a rule, the red droplets are somewhat larger and more irregular in shape than the brown granules. This is particularly true in heart muscle.

What then may be said as to the significance of this pigment? Our own observations and a study of the literature indicate that "ceroid" is found in association with the following conditions:

1. Vitamin E deficiency of rats and other laboratory animals.
2. Dietary cirrhosis in rats, and cirrhosis produced by other means in rats and other laboratory animals.
3. Chronic biliary fistulae and experimental anemia in dogs.
4. Nutritional disorders in man, in which there has been presumptive impairment of absorption by the small intestine.
5. Cirrhosis of the liver and hemochromatosis in man.
6. Focal degenerative lesions.

The "ceroid" deposition which results from dietary lack of vitamin E can be prevented by administration of that vitamin. Although there is no precise knowledge of its derivation or the chemical reactions involved in its deposition, the relation to uncomplicated vitamin E deficiency is established.

In the dietary cirrhosis of rats produced by low protein diets, the presence of large amounts of "ceroid" pigment in the liver has been regarded as a distinctive feature of the hepatic lesion. It has been attributed to break-down products of the damaged liver cells or, more recently, to some derivatives of cod-liver oil in the experimental diets. That an associated vitamin E deficiency may be concerned, has not been considered; the possibility, however, merits discussion.

It is interesting that, with few exceptions, the experimental diets used in the production of hepatic cirrhosis have been obviously deficient in vitamin E. Thus, Lillie, Daft, and Sebrell [1] used a diet in which the only source of tocopherol was 3 per cent of Wesson oil.* Of the diets used by György, only one (diet C 1) contained adequate vitamin E, in the form of 38 per cent hydrogenated cotton-seed oil. Blumberg and Grady [4] found less "ceroid" when the basal diet was supplemented with 3 to 5 cc. of wheat germ oil than was shown by the rats of Blumberg and McCollum [8] which received no vitamin E.

* With an average food consumption of 10 gm. and a value of 64 mg. of tocopherol per 100 gm. of Wesson oil (as determined by Dr. Hans Kaunitz in our laboratory), one may calculate a daily intake of 0.194 mg. of tocopherol. This would be below the normal daily requirement.[20]

Bearing further on this question is a recent paper by Endicott, Daft, and Sebrell,[37] in which they report the production of dietary cirrhosis without "ceroid." Although the authors do not stress the point, it is interesting to note that their rats received a weekly supplement of 3 mg. of alpha-tocopherol. We have examined cirrhotic livers of 21 rats for "ceroid." Acid-fast granules and globules were found in 20, the cirrhosis having been produced by low protein diets, prolonged poisoning with carbon tetrachloride or excess dietary l-cystine, or cysteic acid. In the 5 per cent l-cystine group, acid-fast pigment was strikingly more abundant in 15 rats given a vitamin E-deficient diet than in 15 rats receiving McCollum's stock diet.[38,39]

Further experiments are needed to clear up this question.

"Ceroid" pigmentation of the muscle of the small intestine takes place in dogs with long-standing biliary fistulae, which, as Brinkhous and Warner[22] have shown, also develop muscular dystrophy and atrophy of the testes. It is reasonable to assume that failure to absorb vitamin E from the intestines is the determining factor in producing these characteristic lesions.

In regard to the anemic dogs from Dr. Whipple's laboratory, positive evidence is lacking that vitamin E deficiency was responsible for the "ceroid" deposit in the intestinal musculature. However, the administration of large amounts of cod-liver oil is known to favor destruction of vitamin E; the dogs examined by Nachtnebel[21] received from 10 to 15 cc. of cod-liver oil daily in addition to that incorporated in the salmon bread which formed a large part of their basal diet. Indeed, the only possible source of vitamin E was that which may have been contained in cooked pig's liver.

In the four human cases reported here, in which there was abundant "ceroid" in the intestinal muscles and elsewhere, there is every reason to assume a severe impairment of intestinal absorption. There is, of course, no direct evidence that the ability to absorb vitamin E was in abeyance, but the muscular dystrophy and atrophy of the testes in case 2 present an interesting analogy to the lesions in dogs with biliary fistulae, which, as has been stated, may with some probability be ascribed to deficiency of vitamin E.

In cases of hepatic cirrhosis and hemochromatosis, in which acid-fast pigment was frequently found, one cannot without further knowledge implicate vitamin E deficiency as a factor. If it is proved that lack of vitamin E is responsible for the accumulation of "ceroid" in the hepatic cirrhosis of rats, it will seem logical to ascribe its occurrence in human cirrhosis to a similar deficiency.

Of the occasional finding of "ceroid" about focal degenerative lesions, such as atheromatous plaques, atretic ovarian follicles, or encephalomalacic areas in the brain, one can say little other than to record its occurrence in such situations.

Summary

Acid-fast pigment, "ceroid," has been demonstrated in various tissues including the livers of certain human cases of nutritional disorders and hepatic disease. There is accumulating evidence which suggests a possible relation of this pigmentation to deficiency of vitamin E.

We wish to express our thanks to Dr. Walter W. Palmer and Dr. Allen O. Whipple for permission to use clinical records, and to Mrs. Claudia Schogoleff and Miss Goldie Spierer for technical assistance.

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DESCRIPTION OF PLATE

PLATE 87

FIG. 1. Uterus of rat on vitamin E-deficient diet. "Ceroid" in smooth muscle and phagocytes. × 125.

FIG. 2. Rat 431, 8 months on vitamin E-deficient diet. Anterior horn cell of spinal cord containing mass of "ceroid" in cytoplasm. × 450.

FIG. 3. Rat 3134M, 7 months on vitamin E-deficient diet. "Ceroid" pigment in medulla. × 450.

FIG. 4. Guinea-pig. Atrophic testis following 175 days on vitamin E-deficient diet. "Ceroid" within atrophic tubules. × 450.

FIG. 5. Case 1 (autopsy 14033). Small intestine showing brown pigmentation of muscularis.

FIG. 6. Case 1. Small intestine. "Ceroid" pigment in muscle cells and phagocytes. × 100.

FIG. 7. Case 1. Uterus. "Ceroid" in muscle fibers. × 450.

FIG. 8. Case 1. Ovary. Phagocytes containing "ceroid" in stroma. × 450.

FIG. 9. Case 2 (autopsy 12281). Skeletal muscle, showing atrophy. "Ceroid" in myocytes and phagocytic cells. × 450.

FIG. 10. Cirrhosis of liver (human). "Ceroid" in liver cells and phagocytes. × 450.

FIG. 11. Hemochromatosis (autopsy 14460). "Ceroid" in pars nervosa of pituitary gland. × 450.

FIG. 12. Cirrhosis of liver (autopsy 14342). Seminal vesicle. "Ceroid" in epithelial cells and fibrocytes of stroma. × 450.

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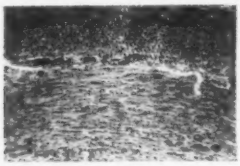

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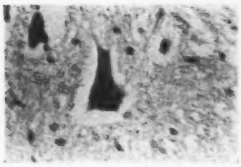

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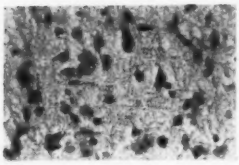

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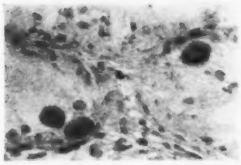

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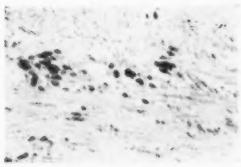

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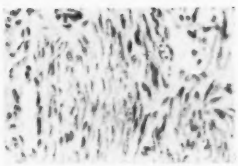

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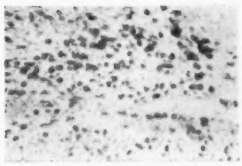

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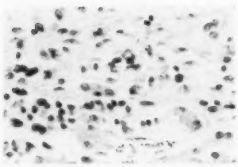

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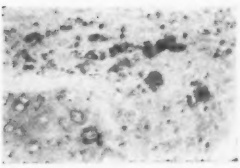

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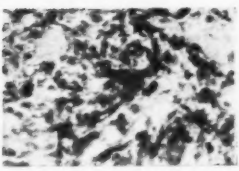

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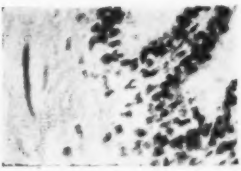

STUDIES ON CHANCROID

I. Observations on the Histology with an Evaluation of Biopsy as a Diagnostic Procedure *

Walter H. Sheldon, M.D., and Albert Heyman, M.D.

(From the Departments of Pathology and Medicine (Clinic for Genitoinfectious Diseases), Grady Memorial Hospital and Emory University School of Medicine, Atlanta, Ga.)

This report is the first in a series of studies dealing with the clinical, bacteriologic, and histologic aspects of chancroidal infection. In this paper the histologic appearance of chancroid is described and biopsy as a diagnostic procedure is discussed.

There is definite need for a simple and accurate laboratory method by which the diagnosis of chancroid can be established.[1,2] This has been emphasized recently by the statement from the Surgeon General of the United States Army that the usual laboratory tests are not recommended.[3] The Ducrey skin test was thought to be diagnostic, but Knott and his associates [4] have shown that this test is of limited value. Auto-inoculation is not generally used in the diagnosis, since it produces a new infection. The identification of the Ducrey bacillus is the most accurate method of diagnosis but the demonstration of the organism in direct smears of the lesion has many pitfalls,[5] and isolation by culture is not generally attempted.

In this study cultures were taken from all lesions from which tissue had been obtained for biopsy. The identification of the Ducrey bacillus confirmed the histologic diagnosis in our cases and offered, at the same time, an opportunity to compare the usefulness of culture and biopsy. To our knowledge no similar studies have been reported in which a large group of cases were investigated concurrently by histologic and bacteriologic methods.

Method of Study

The histologic study is based upon 45 specimens taken for biopsy, which were diagnosed as chancroid. They occurred among 59 specimens taken from 125 patients who had been selected for a special study of lymphogranuloma venereum and chancroid. These patients were subjected to a number of diagnostic procedures which included skin tests, microscopic examination of stained smears, darkfield preparations, lymphogranuloma complement-fixation tests, serum protein determinations, biopsy, auto-inoculation, culture, and inoculation of mice and chick embryos.

* Aided by a grant from the Venereal Disease Division of the United States Public Health Service.

Received for publication, April 30, 1945.

Tissue specimens were taken without anesthesia with a Gaylors biopsy forceps from the base of the ulcer, usually with little pain. Care was taken to avoid the adjacent skin, and bleeding was easily controlled by local pressure. The specimens averaged 3 mm. in diameter; they were fixed immediately in Zenker's fluid containing 5 per cent glacial acetic acid. Mallory's phloxine-methylene blue stain was used routinely, but in some instances Giemsa's and Gram's stains were also used. The histologic diagnoses were made within 24 hours, and were returned before the results of the cultures were known.

Cultures were taken from the primary lesion or from the auto-inoculation in every case. A modification of the Teague and Deibert method [6] was used and the culture was reported as positive only when the typical tangled chains of gram-negative coccobacilli, the Ducrey bacillus, were found. A discussion of the technic of culture is to be reported elsewhere.

There were 28 male and 17 female Negro patients in this series. The lesions varied in duration from 2 days to 4 weeks. In two instances the lesions occurred in the upper vagina or on the cervix, while in the remainder the lesions were located on the external genitalia.

Histologic Observations

Little information on the histologic appearance of chancroid is found in the standard textbooks of pathology, although a description of some features is given by Moore.[7] He described an intra-epidermal abscess extending into the dermis, perivascular infiltration by polymorphonuclear leukocytes and a few plasma cells, dilatation of blood vessels and lymphatics, and marked acanthosis of the epithelium at the edge of the ulcer.

According to McCarthy,[8] the "most typical histologic picture" is an ulcer which is generally confined to the upper corium, but may extend into the subcutaneous tissues. There is moderate acanthosis of the epithelium. Lymphocytes and plasma cells participate to an approximately equal degree in the diffuse and dense inflammatory infiltration. Polymorphonuclear leukocytes predominate on the ulcerated surface. The inflammatory cellular infiltration blends with the adjoining tissue where it forms perivascular cuffs. The blood vessels and lymphatics are dilated and the former show marked endovasculitis and perivasculitis.

Pund, Greenblatt, and Huie [9] described similar changes and stressed the marked swelling of the endothelium which obstructs the capillaries and leads to necrosis. They considered the vascular changes as "characteristic and responsible for the superficial necrosis and unhealthy granulation." Von Haam [10] felt that these changes represent only a

superimposed fusospirochetal infection. In his observations he noted the absence of any vascular lesions and stated that the histologic picture of chancroid is characterized by the absence of specific changes. Greenblatt [5] considered the histologic changes as suggestive of chancroid, but not sufficiently distinct to permit the diagnosis on the basis of biopsy alone.

Our observations are based upon small specimens, most of which were taken for biopsy from fully developed or persistent lesions. No attempt will be made here to follow the sequences of formation in this lesion. However, the comparatively large number of specimens in this series, as well as 65 additional specimens of various genital lesions obtained from sources outside this study, made possible the development of certain general concepts. Our conclusions were further supported by independent bacteriologic studies of the same lesions.

The following description represents a composite picture as reconstructed from the study of numerous specimens, and refers to an active lesion of about 2 to 3 weeks' duration.

The lesion rarely extends more than 2 or 3 mm. in depth and under low magnification shows two or three zones which blend into each other (Fig. 1). There is generally a rather shallow surface zone of necrotic tissue representing the base of the ulcer. Below this is a fairly wide layer of edematous tissue with numerous blood vessels which are prominent and dilated. Their walls are thin and it is obvious that they are newly formed. In properly oriented sections the blood vessels display a vertical or palisade-like arrangement in their relation to the ulcerated surface. This zone leads into the deep layer in which there is a marked and diffuse cellular infiltration. Here the blood vessels, although still numerous, are less conspicuous and without any particular arrangement. The cellular infiltration fades into the adjacent edematous tissue where it persists around the dilated blood vessels.

On close observation the surface zone consists only of necrotic tissue, red blood cells, some fibrin and large numbers of neutrophilic polymorphonuclear leukocytes. Microorganisms are common and persistent search may reveal gram-negative coccobacilli between the cells of the surface zone, which are morphologically consistent with the Ducrey bacillus.

The mid-zone of the lesion is cellular. It is evident that the majority of cells in this zone are endothelial cells in varying stages of proliferation (Fig. 2). These outnumber all other cells. They are seen as apparently single elements or in small groups, as solid cords or buds originating from a capillary, and as already partially patent channels. Where the mid-zone joins the surface layer, the endothelial cells are

undergoing necrosis (Fig. 3); elsewhere they develop into capillaries. No microorganisms can be seen within these cells.

Marked endothelial proliferation is also seen in both the pre-existing and newly formed vessels in which the endothelium tends to encroach upon the lumen. The process involves arterioles, veins, and capillaries (Fig. 4). Fibrinoid degeneration of the vessel wall, margination and infiltration by neutrophilic polymorphonuclear leukocytes, and sometimes thrombosis are seen near the surface layer (Figs. 3 and 5).

The interstitial connective tissue is edematous. The lack of significant proliferation of fibroblasts in the presence of the marked endothelial overgrowth is striking (Fig. 2). Toward the surface a moderate number of neutrophilic polymorphonuclear leukocytes are present, while plasma cells appear in the deeper portions.

The deep zone of the lesion displays a marked infiltration by plasma cells and less numerous lymphocytes. This infiltration is diffuse and not particularly perivascular. The numerous blood vessels show well formed and thick walls. Endothelial proliferation is less marked and is largely confined to the lumen of the vessels. No degeneration of vessel walls with leukocytic infiltration or thrombosis is encountered.

The tissue surrounding the lesion is edematous and shows some fibroblastic proliferation. The blood vessels are dilated and their endothelium is prominent. There is some perivascular cuffing with lymphocytes and plasma cells. The lymphatics are markedly distended with granular eosinophilic material, some fibrin, and varying numbers of white blood cells. Occasionally, fibrin thrombi are found in the lymphatics which then may show appreciable endothelial proliferation.

The epidermis at the edge of the ulcer is necrotic and more peripherally shows edema, acanthosis, and some polymorphonuclear leukocytic infiltration. There is no repair. The inflammatory cellular infiltration extends for some distance beneath the epidermis on the sides of the ulcer.

In lesions of about 1 week's duration the findings are similar. Here, however, the surface zone of necrosis and ulceration is wide as compared with the mid-zone or the deep layer. The mid-zone displays the same striking endothelial overgrowth without proliferation of fibroblasts which has already been described. Necrosis of the vessel walls with leukocytic infiltration and thrombosis is frequent. The deep zone is poorly developed and there is relatively slight infiltration by plasma cells and lymphocytes.

In lesions older than 3 weeks, or when there is no further evidence of spreading, lymphocytes increase in the deep zone and eventually

outnumber the plasma cells. Sometimes distinct lymphoid follicles are formed. Plasma cells and, later, lymphocytes extend upward into the mid-zone. Healing takes place by proliferation of fibroblasts extending from the deep zone between the numerous blood vessels of the mid-zone (Fig. 6). Collagen is deposited, and some of the blood vessels, pinched off by connective tissue, undergo atrophy. By now the vessels have acquired thick walls. An increased number of endothelial cells line the vessels, but endothelial proliferation has largely subsided (Fig. 7). The base of the ulcer is filled in this manner and the epithelium at the edge of the lesion regenerates.

The same picture is seen not only in the primary lesion, but also in tissue from an area of auto-inoculation or from the wall of a ruptured bubo.

When the specimen includes tissue from all parts of the lesion the recognition of chancroid is not difficult. In many instances, however, the specimen is taken from the base of the ulcer and may show only the surface and mid-zone, with little of the deep layer and nothing from the edge or the surrounding tissues included. Even then the findings are sufficient to permit the diagnosis.

The demonstration of gram-negative coccobacilli in the section is not considered diagnostic, since it is subject to the same uncertainties as the direct smear.

Results

The histologic diagnosis of chancroid was made in 45 of 59 specimens taken for biopsy. The diagnoses in the remaining 14 cases were: lymphogranuloma venereum (7), granuloma inguinale (2), syphilis (2), and nonspecific inflammatory process (3). Since this investigation was intended to study chancroid and lymphogranuloma venereum, the preponderance of chancroidal infections in our biopsy material was to be expected. The routine material obtained from sources other than this series showed the usual incidence of venereal diseases.

The diagnosis of chancroid was made by biopsy in 45 cases, 35 of which were confirmed by culture. Biopsy yielded the correct diagnosis in all but one of the cases proved by culture. In this instance the Ducrey bacillus was cultured from the lesion, but biopsy showed granuloma inguinale with Donovan bodies.

There were 10 cases in which the culture for the Ducrey bacillus was negative but biopsy revealed chancroid. In all of these 10 cases the clinical picture was consistent with the diagnosis of chancroid.

Three patients in the group of 59 appeared clinically to have chancroid, but the specimens for biopsy showed only a nonspecific picture and the cultures were negative.

Comment

The histologic diagnosis of chancroid is based upon a number of findings, which taken individually are not characteristic but when found together may permit the diagnosis. There is the general architecture of the lesion consisting of an ulcerated surface and one or two deeper layers. The blood vessels display one of the most striking features. There is marked endothelial proliferation which in its diverse aspects occurs predominantly in the mid-zone. Here the endothelium in the various stages of overgrowth outnumbers all other cells. In the same area the lack of appreciable fibroblastic proliferation constitutes another important finding. The palisading of blood vessels, with degeneration of their walls and occasionally thrombosis, is part of the peculiar vascular pattern. There is finally the dense infiltration of the deep zone by plasma cells and lymphocytes with a gradual transition into the surrounding tissues.

The histologic picture can be differentiated from that of other genital lesions. It can be distinguished with reasonable accuracy from nonspecific inflammatory processes by following the criteria which have been outlined. Syphilis must be excluded, but its histologic picture is sufficiently well known to separate it from chancroid. Little is known about the histologic picture of the primary lesion in lymphogranuloma venereum, but in our experience it does not seem to bear any resemblance to chancroid. Granuloma inguinale is easily ruled out, for the general pattern is different and that diagnosis can be established by demonstrating the Donovan bodies.

The Ducrey bacillus was not cultured in 10 of the 45 cases in which a diagnosis of chancroid was made by biopsy. Two of these 10 lesions were on the upper vaginal mucosa and cervix, while in another instance the material was from a ruptured bubo. Because of the location and character of these lesions, the cultures were heavily contaminated. It has been our experience that the presence of other bacteria interferes with the growth of the Ducrey bacillus. The clinical appearance of the remaining 7 cases was entirely consistent with chancroid.

In this study we have encountered but one instance of mixed venereal infection in the same lesion. In this case, as already mentioned, the Ducrey organism was cultured from the lesion but biopsy showed the histologic picture of granuloma inguinale with typical Donovan bodies and no evidence of chancroid. Concurrent lymphogranuloma venereum was noted in 2 cases. In 5 other cases syphilis developed while the patients were under observation.

The diagnosis of chancroid by biopsy appears to have certain advantages. In our experience, securing tissue for biopsy is a simple procedure and does not interfere with healing of the lesion. For a variety of reasons the diagnosis is made more often by histologic examination than by culture. Although one tissue specimen is usually sufficient, two or three attempts to culture the lesion were sometimes necessary before the Ducrey bacillus could be identified. In fact, in 2 cases the Ducrey organism was obtained only from auto-inoculation lesions and not from the primary. Examination by biopsy seems particularly useful for those lesions which, because of location or long duration, are heavily contaminated by other bacteria. Present cultural methods usually require more than 24 hours of incubation, while our histologic diagnoses are routinely returned within this period. Securing tissue for biopsy, however, is a painful procedure in extremely small or early lesions and in such cases it is not a practical procedure. Fortunately, diagnosis by culture and smear is usually possible under such circumstances because of fewer contaminating bacteria.

In the course of this study the clinical diagnosis was changed in a number of cases by biopsy. Histologic examination not only confirmed other laboratory procedures but was also valuable when other methods failed. Early carcinoma, granuloma inguinale, and primary syphilis have been diagnosed on histologic evidence alone.

Conclusions

The histologic character of chancroidal infection was studied in specimens taken for biopsy from 45 patients. They formed part of a series of 125 cases selected for a special study of chancroid and lymphogranuloma venereum. In 35 of these 45 cases, the histologic diagnosis of chancroidal infection was confirmed by culture of the Ducrey bacillus obtained from the same lesions. The organism was not cultured from the other 10 cases, in which, however, the clinical picture was consistent with the diagnosis of chancroid.

The histologic picture of chancroid is sufficiently distinct to permit diagnosis and to differentiate this condition from other genital lesions. Useful features are the zonal character of the inflammatory reaction, the marked endothelial proliferation in the mid-zone, the meager fibroblastic response at the same level, and the dense infiltration with plasma cells and lymphocytes in the deepest zone. The advantages of biopsy in the diagnosis of chancroidal infection are such that this procedure is suggested as a practical diagnostic tool.

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DESCRIPTION OF PLATES

PLATE 88

FIG. 1. The general pattern of the lesion. The shallow ulcerated surface zone is followed by the mid-zone and by the deep layer. In the mid-zone there are numerous blood vessels with some palisading. Some vessels show degeneration of their walls. Phloxine-methylene blue stain. × 80.

FIG. 2. Endothelial cells in varying stages of proliferation in the mid-zone. Endothelial cells outnumber all others, most of which are polymorphonuclear leukocytes. Phloxine-methylene blue stain. × 600.

FIG. 3. The transition of the mid-zone into the base of the ulcer where polymorphonuclear leukocytes predominate. The small blood vessel in the center shows degeneration of the wall and early thrombus formation. Below and to the right of the vessel is a small group of endothelial cells undergoing necrosis. Phloxine-methylene blue stain. × 550.

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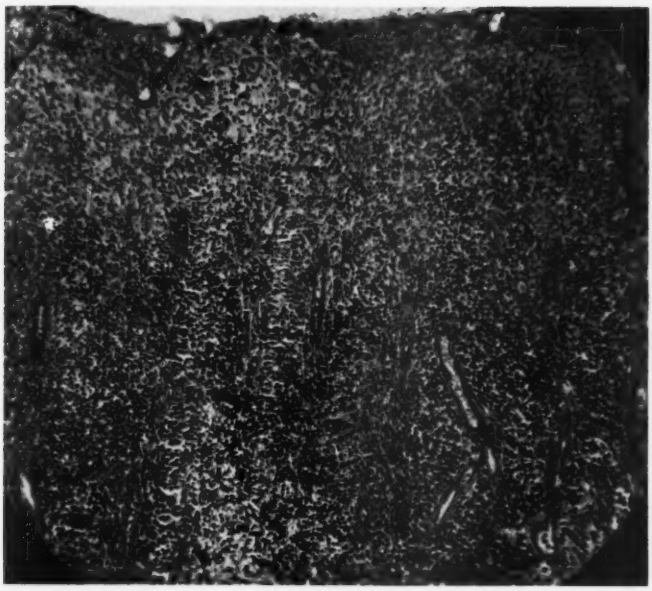

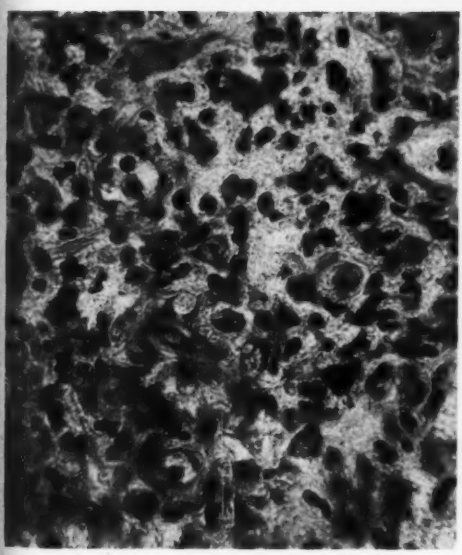

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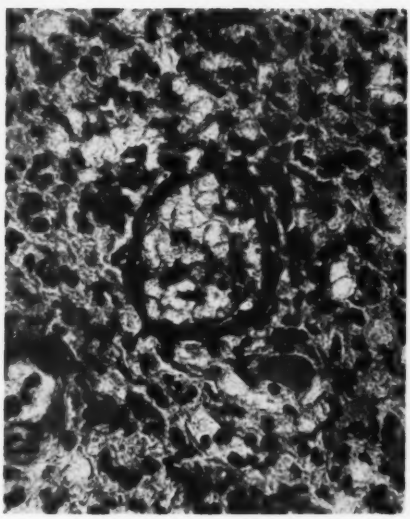

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Sheldon and Heyman

Diagnosis of Chancroid by Biopsy

PLATE 89

FIG. 4. The numerous blood vessels in the mid-zone of the lesion showing proliferation of their endothelial lining. Phloxine-methylene blue stain. × 230.

FIG. 5. Another small artery of the mid-zone near the base of the ulcer with leukocytic infiltration of the wall and beginning fibrinoid degeneration. Phloxine-methylene blue stain. × 550.

FIG. 6. The mid-zone in a healing lesion of 28 days' duration. Of note are the thick walls of the blood vessels, the increase in interstitial connective tissue, and the lymphoid and plasma cell infiltration. Phloxine-methylene blue stain. × 200.

FIG. 7. Higher magnification of the lesion shown in Figure 6. Some vessels have become atrophic and consist only of small whorls of connective tissue. Phloxine-methylene blue stain. × 420.

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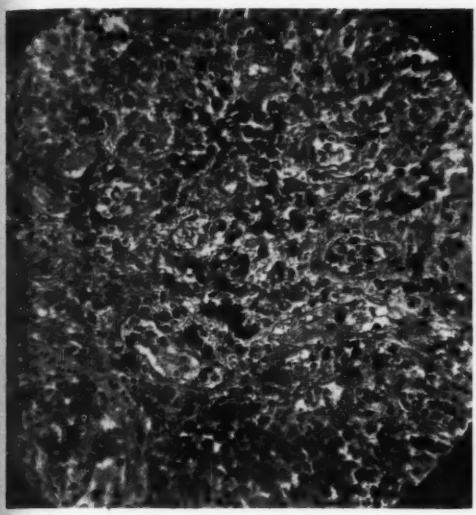

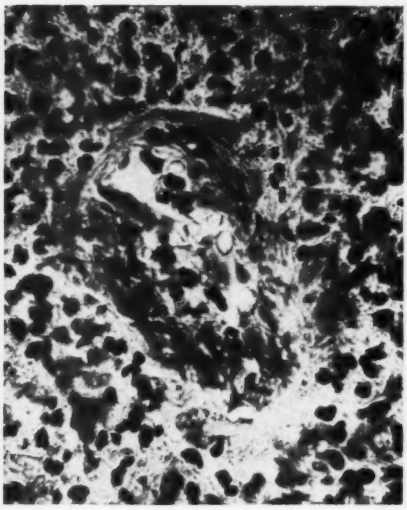

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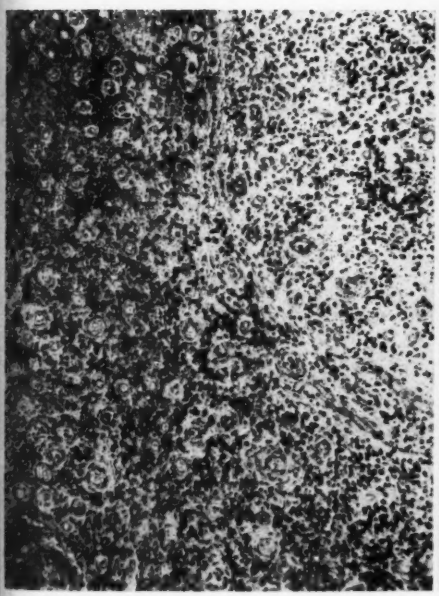

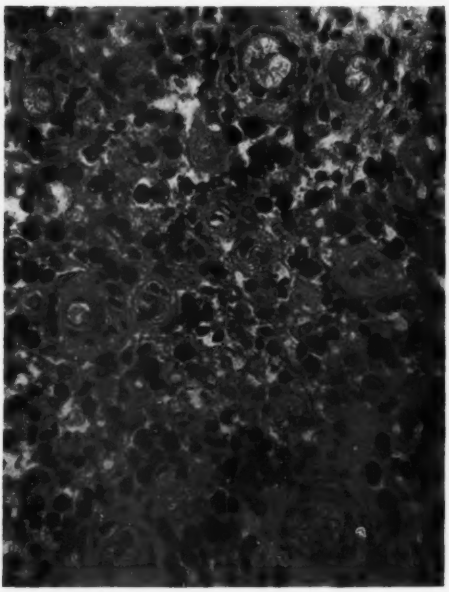

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Sheldon and Heyman Diagnosis of Chancroid by Biopsy

THE PROBLEM OF HUMAN TOXOPLASMA CARRIERS *

ALFRED PLAUT, M.D.

(From the Department of Pathology, Beth Israel Hospital, New York, N.Y.)

Newborn children can exhibit the fullfledged picture of toxoplasmic encephalitis while the mother is healthy. Therefore, it stands to reason that the mother must have harbored the parasite at least temporarily. An editorial in the *Journal of the American Medical Association*,[1] closing with the words "Are there carriers of Toxoplasma?", put a question every worker in the field must have asked himself, and the literature contains pertinent remarks.

Two observations, made in the course of routine autopsy work, illustrate the difficulty of the problem, and they may stimulate the interest of pathologists.

FIRST OBSERVATION

The first observation was made upon a 14-year-old white boy who had succumbed to sub-chronic yellow atrophy of the liver. His previous history was noncontributory. Five months before admission jaundice was ushered in by an attack of abdominal pain. The stools were light in color. For the ensuing 2 months the jaundice varied in intensity but never disappeared. About 2 months before admission a physician noted swelling of the abdomen and enlargement of the liver and spleen. Jaundice remained; there was neither pain nor itching; the urine is said not to have been dark. Appetite remained good. During the week before admission the boy vomited a few times. Legs and ankles began to swell. On admission he was deeply jaundiced and drowsy. The abdomen was distended, doughy, and no masses could be palpated. The tonsils were large and the nostrils contained dry blood. The patient declined rapidly. Jaundice deepened; the temperature rose to 105° F., and he became disoriented. There was bleeding from the right tonsil and bloody vomitus. An agglutination test for Weil's disease was negative. The clinical diagnosis was subacute atrophy of the liver (Dr. I. W. Held). Death occurred on the tenth hospital day. This boy had lived on Long Island, near the seashore. There were rats about, but he did not recall having been bitten.

At *autopsy*, severe jaundice was noted. The abdominal cavity contained 2000 cc. of thin yellow fluid with some flakes of fibrin. The spleen was large and soft (440 gm.). The liver (1290 gm.) was nodular; the nodules measured up to 1 cm. and were bright yellow with hemorrhagic areas. Between the nodules the liver tissue was sunken, yellowish pink, without markings. There were partly ruptured varices above the cardia, and some lung areas were filled with blood. The third portion of duodenum and adjoining loops of jejunum were phlegmonous. The skull could not be opened.

Microscopically, two-fifths of the liver sections consisted of islands of regeneration, the remainder being connective tissue with the usual

* Received for publication, July 7, 1945.

trabecular and pseudotubular epithelial structures. Necrosis and hemorrhage were not conspicuous. No microorganisms could be demonstrated in liver or spleen. Levaditi stain was negative for organisms. Hemolytic streptococci were cultured from a parapancreatic lymph node. The anatomical diagnoses were: Sub-chronic yellow atrophy of liver, with beginning cirrhosis; ruptured esophageal varices; aspiration of blood; phlegmon of duodenum and jejunum; swelling of spleen.

In the routine section of myocardium, under low magnification, a blue dot caught the eye. It was trapezoidal in shape, the base measuring 48 μ, the upper horizontal and the sides, 20 μ. This mass was situated in a myocardial fiber which, correspondingly, was thickened to at least twice its diameter. Otherwise it did not differ from surrounding fibers, and there was no tissue reaction (Fig. 1). This mass consisted of countless, closely packed, very small, irregularly round and ovoid appearing particles (Figs. 2 and 3). Between the particles one did not see the red of the muscle fiber but an indefinite purplish pink. The whole structure had a fairly distinct outline, and did not occupy the whole thickness of the myocardial fiber. In the same section, 1 cm. away, a similar structure was found (Fig. 4). Its surroundings also were entirely normal. Its shape was less regular, somewhat that of a triangle, the base measuring 45 μ, the sides 32 and 30 μ. The granules in this structure stained a little darker with hematoxylin. They were round or round-ovoid. Since they obviously were distributed irregularly and since no long forms were found, one may assume that they all were either spherical or round-ovoid. The average diameter of each granule was 1 μ (object micrometer, oil immersion). The granules were not homogeneous; they contained dark-staining and light-staining areas. On changing the focus one gained the impression that the dark-staining areas were more peripheral. (This subdivision of the particles into cytoplasm and chromatin is not brought out in the photographs.) No further parasites were found in numerous sections from myocardium and other organs, especially spleen, lymph nodes, and liver. The brain, as mentioned, was not available.

The slide and the photographs were submitted to seven specialists. The verdict of two of them was "most likely Toxoplasma." The third one first refused to make a diagnosis but joined in after others had favored Toxoplasma. One parasitologist, on seeing the photographs, thought of Toxoplasma first; he included Sarcosporidia and *Trypanosoma cruzi* for differential diagnosis. From the slide he diagnosed sarcosporidiosis. The next one diagnosed either *Trypanosoma cruzi* or *Leishmania donovani;* he considered the particles too small for Toxo-

plasma, and he felt sure they were neither Sarcosporidia nor Histoplasma. Another, who saw only the photographs, wrote "Toxoplasma or Sarcosporidia," and the last one said, "nothing which is incompatible with Toxoplasma" (photographs only seen).

Comment on First Observation

Considering the divergent opinions of the experts, it seems futile to compare these findings with numerous pictures from the literature. The similarity of my Figures 2 and 3 with Figures 1 and 2 in the paper by Kean and Grocott [2] will strike the readers of this *Journal*. Dr. Kean and I had seen the photomicrographs of each others cases in 1944. There is a similarity also with the photographs labelled sarcosporidiosis in the paper by Gilmore, Kean, and Posey.[3] The reasons given by these authors for calling the organisms Sarcosporidia might be applied—in a certain degree—to Figures 1 and 2. Only one of the specialists thought that the organisms had something to do with the clinical course; he diagnosed Leishmania and considered the whole case kala-azar. It is for this reason that the history of the case is given in some detail. No parasites could be found, however, in other organs.

Second Observation

The structure pictured in Figures 5 and 6 was found in the routine section of myocardium of a 66-year-old woman. This patient died of peritonitis following a two-stage operation for intussusception, caused by a large, partly carcinomatous polyp. There was no tissue reaction that could be ascribed to the presence of this microorganism. The remainder of the autopsy findings were noncontributory. A detailed description seems unnecessary. It might be useful to compare Figure 5 with Figure 432 in Wenyon's Protozoology (1926).[4] Figure 432 is designated as Toxoplasma.

This slide (or the photographs) were submitted to five specialists. Their opinions were as follows:

1. In all probability, Toxoplasma.
2. No opinion; has seen it once in the human brain.
3. No opinion; has seen it in the brain.
4. Contaminant from water or a similar source; has seen it in brain sections on several occasions.
5. Toxoplasma.

The fact that such organisms have been seen in the brain, and now once in the myocardium, but not in other organs, in my opinion does not speak in favor of a contaminant.

Summary

1. In two autopsies protozoon-like structures were found in the myocardium. There was no tissue reaction.

2. It is difficult, if not impossible, to classify such isolated findings. Toxoplasma and Sarcosporidia are the most likely diagnoses in the first observation. There is only a possibility that we may deal with Toxoplasma in the second observation.

3. The study and discussion of such occurrences are important because we must find the human carriers of Toxoplasma.

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3. Gilmore, H. R., Jr., Kean, B. H., and Posey, F. M., Jr. A case of sarcosporidiosis with parasites found in heart. *Am. J. Trop. Med.*, 1942, **22**, 121–125.
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Description of Plate

Plate 90

Fig. 1. Parasite, perhaps Toxoplasma, in the myocardium of a 14-year-old boy. No tissue reaction. Hematoxylin and eosin stain. × 360.

Fig. 2. Higher magnification of Figure 1. × 960.

Fig. 3. Higher magnification of Figures 1 and 2. × 1450. The parasite does not occupy the whole thickness of the muscle fiber.

Fig. 4. Another parasite from the same section as shown in Figures 1, 2, and 3. × 1450.

Fig. 5. Nucleated, ovoid organisms, one end pointed, radially arranged. From the myocardium of a 66-year-old woman. No tissue reaction. Hematoxylin and eosin stain. × 600.

Fig. 6. Higher magnification of the smallest rosette from Figure 5. × 1450.

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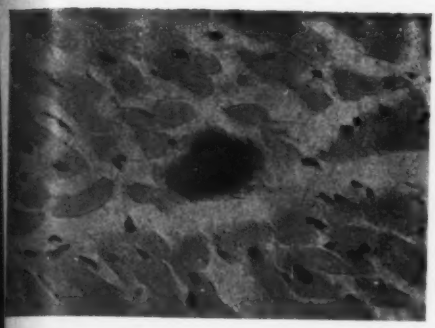

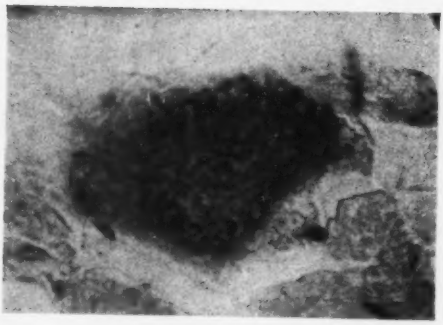

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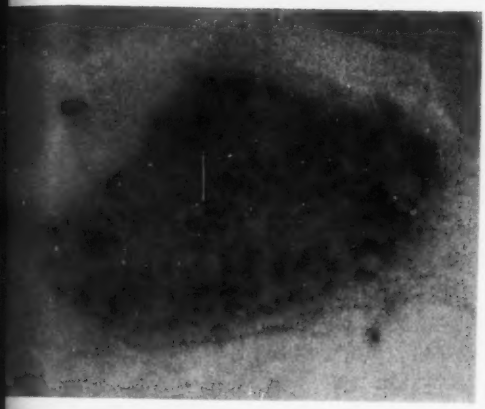

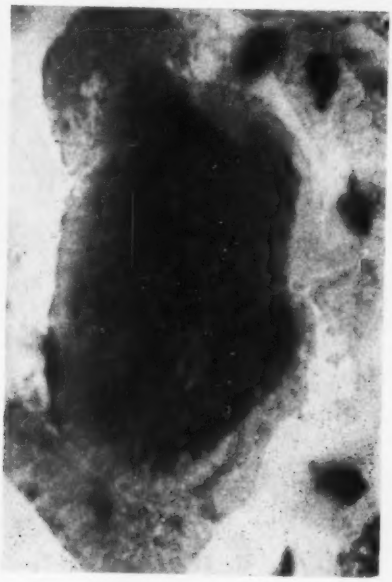

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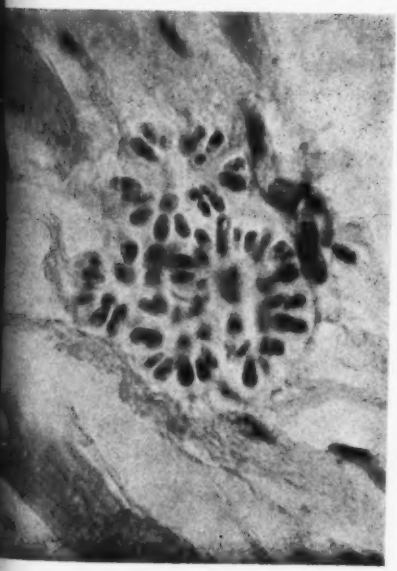

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